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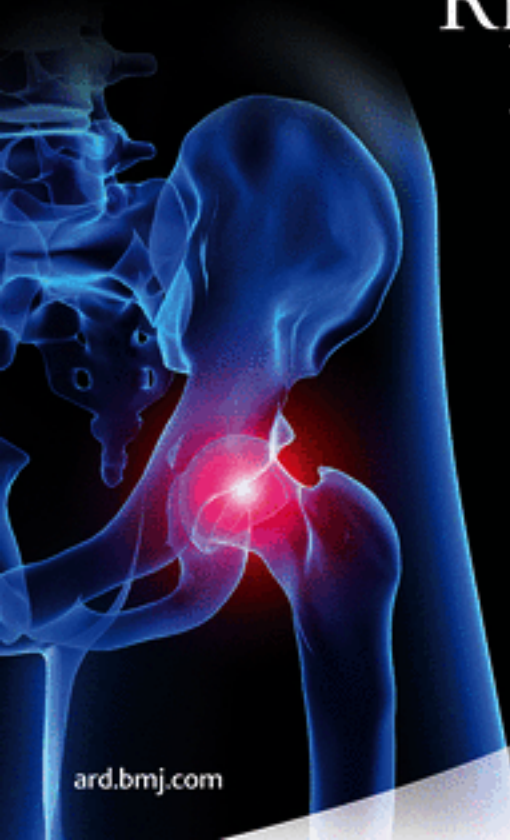

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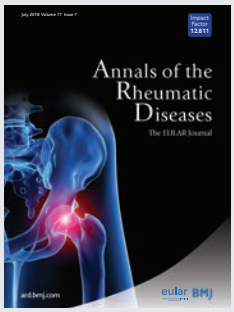

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Adalimumab for childhood onset uveitis

A V Ramanan,[1,2] Catherine Guly[2]

Juvenile idiopathic arthritis (JIA)-associated uveitis is the most common cause of paediatric uveitis and is associated with significant visual morbidity.[1] Despite considerable improvements in the treatment of JIA, most trials of biological agents in JIA excluded children with uveitis for methodological reasons. This has meant a limited evidence base and availability of biological therapies for paediatric uveitis. The ADJUVITE study[2] published in this journal and the recently published SYCAMORE study[3] both provide much needed evidence for use of biologics in children with uveitis.

The ADJUVITE study[2] randomised 32 patients with childhood onset anterior uveitis and an inadequate response to topical steroid and methotrexate (MTX), based on a laser flare photometry (LFP) reading of ≥30 photon units/ms, to fortnightly adalimumab or placebo. The primary outcome was response to treatment at the end of month 2, defined as a reduction of at least 30% of ocular inflammation on LFP with no worsening of anterior chamber cells or flare according to Standardised Uveitis Nomenclature (SUN) criteria.[4] In the adalimumab group, 9/16 patients had a 30% reduction in flare on LFP compared with 3/15 in the placebo group, which was statistically significant, but as the authors acknowledge should be interpreted with some caution as the CI included 1. There was no significant reduction in anterior chamber cell scores.

This small study, following on from the SYCAMORE trial,[3] which was stopped early due to evidence of efficacy of adalimumab for JIA-associated uveitis, provides further support for the use of adalimumab in childhood uveitis.

However, there are striking differences between the ADJUVITE and SYCAMORE studies. SYCAMORE recruited 90 children with JIA-associated uveitis who had failed treatment with topical or systemic glucocorticoids and MTX and had active uveitis with ≥1+ anterior chamber cells based on the SUN criteria. The primary outcome of SYCAMORE was time to treatment failure based on the SUN anterior chamber cell grading score. The ADJUVITE participants had lower cellular activity at baseline than those in the SYCAMORE study; indeed, 15 patients (48%) in ADJUVITE with a cell count of 0–0.5+ would not have been eligible for entry to the SYCAMORE study. Despite the lower cell counts, eyes in the ADJUVITE study had greater morbidity with 76% of eyes having posterior synechiae compared with 27% in the SYCAMORE study.

These trials leave us with important questions to answer over which children should be offered adalimumab, how we should monitor inflammatory activity in clinical practice and what we should measure in clinical trials in paediatric uveitis.

One of the limitations of the SYCAMORE study was the exclusion of children with idiopathic uveitis, which ADJUVITE does try to address. Although only two children with idiopathic uveitis are included in the study, it is important to acknowledge that idiopathic chronic anterior uveitis in children has a similar course to JIA-associated uveitis.[5] The trial results cannot be generalised to posterior segment uveitis, where evidence for biological treatment remains limited.

Grading of uveitis activity on slit-lamp examination was standardised by the SUN working group in 2005 with anterior flare and cells graded on a scale of 0–4 (4). Flare is the cloudy appearance of aqueous humour due to accumulation of protein and the anterior chamber cell score is based on the number of cells seen in a 1×1 mm slit-lamp beam. While the anterior chamber cell grading score is used routinely in clinical practice to determine disease activity and to make management decisions, the anterior chamber flare score is less discriminatory with clustering at the lower end of the score range.[3] Laser flare photometry offers a more objective reproducible measure of flare with a greater range of readings, but requires specialist equipment and may be unreliable in certain situations such as a shallow anterior chamber or an eye with a mature cataract.[6]

Ocular inflammation at baseline, whether measured by aqueous flare ≥1+, LFP ≥20 or anterior chamber cells ≥1+, has been shown to be predictive of vision loss and ocular complications in childhood uveitis,[7–9] although flare at presentation tends to be a stronger predictor of visual loss.[10 11] Anterior chamber cells during follow-up of ≥0.5 to ≥1+ are associated with loss of visual acuity in eyes with JIA uveitis[7 10] with a progressive increase in risk of sight loss as cell counts increase.[10] Conversely, low SUN cell counts and flare on LFP at baseline and during follow-up is associated with lower rates of complications.[9 11] However, cell counts and LFP readings do not always correlate and children may have high flare readings but low cell counts, as was seen in the ADJUVITE study, and the reverse has also been reported[11] (see table 1).

ADJUVITE has shown that flare may reduce with adalimumab treatment in patients with low cellular activity, but there remains uncertainty over whether anterior chamber flare is always representative of active inflammation and if treatment of flare alters the outcome of the disease.[12]

The failure of ADJUVITE to show a significant improvement in cellular activity is likely to be due to the low cellular activity at baseline and the short interval to the primary end point at only 2 months. In most trials of biological agents in JIA, it has taken 3–4 months to show a clinical response to the treatment.[13 14] It is very likely that chronic anterior uveitis, like arthritis, will need therapy for at least 3 months before one could state that the agent is not effective.

Adalimumab is costly with the potential for adverse events[3] and unknown long-term risks. The short follow-up period in ADJUVITE limits conclusions on the safety of adalimumab. However, both ADJUVITE and SYCAMORE[3] have shown that adalimumab is well tolerated in children with uveitis, and more children were withdrawn from SYCAMORE for MTX intolerance than for problems related to adalimumab. This is important as emotional reactions to treatment have a significant impact on quality of life in JIA-associated uveitis.[15] Duration of therapy is also an important consideration in children, and we need longer term outcomes to determine the optimal time to intervene and withdraw biological therapy in childhood uveitis.

ADJUVITE and SYCAMORE have approached the same research question in very different ways, despite broadly following published proposed outcome measures for clinical trials in JIA-associated uveitis.[16] We should look to

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Table 1 Measurement of anterior chamber inflammation in uveitis

SUN anterior chamber cells	Laser flare photometry
No additional equipment	Laser flare photometer and trained operator
Some patient cooperation required	Greater patient cooperation required
Sign of inflammation	Sign of inflammation±damage
Semiquantitative, limited range of measurements, less reproducible	Quantitative, fully objective, greater range of measurements, reproducible
Dependent on experience	Measurements affected by time of day, age, mydriasis, cataract[6]
Cells ≥0.5–1+ associated with vision loss and ocular complications[7 10] and <1+ cells with reduced complications[9]	Elevated flare associated with vision loss and ocular complications[12] and flare <20 photon units/ms with reduced complications[11]
Not validated for paediatric uveitis but widely used in clinical practice and in adult uveitis trials[17 18]	Not validated for paediatric uveitis. Limited use in clinical practice and research

SUN, Standardised Uveitis Nomenclature.

validating scores of inflammation for children and forming a consensus on the management of paediatric uveitis trials for the future.

The advent of several new agents (biologics and JAK inhibitors) does bring the prospect of more trials in children with uveitis. It is important that future trials include both JIA associated and idiopathic uveitis children in studies. ADJUVITE and SYCAMORE have firmly provided the evidence for adalimumab in MTX-refractory uveitis. This would make placebo-controlled or randomised placebo phase design studies less feasible or palatable for patients and clinicians. We believe adaptive designs involving small numbers of patients in open-label or comparator studies is the 'need of hour' to achieve better outcomes in the management of children with refractory uveitis.

Correction notice This article has been corrected since it published Online First. In the last line of paragraph 4, 'APTITUDE' was changed to 'SYCAMORE' in the sentence "Despite the lower cell counts, eyes in the ADJUVITE study had greater morbidity with 76% of eyes having posterior synechiae compared with 27% in the SYCAMORE study."

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REFERENCES

1 Sen ES, Dick AD, Ramanan AV. Uveitis associated with juvenile idiopathic arthritis. *Nat Rev Rheumatol* 2015;11:338–48.
2 Quartier P, Baptiste A, Despert V, *et al*. ADJUVITE: a double-blind, randomised, placebo-controlled trial of adalimumab in early onset, chronic, juvenile idiopathic arthritis-associated anterior uveitis. *Ann Rheum Dis* 2017. doi: annrheumdis-2017-212089.
3 Ramanan AV, Dick AD, Jones AP, *et al*. Adalimumab plus methotrexate for uveitis in juvenile idiopathic arthritis. *N Engl J Med* 2017;376:1637–46.
4 Jabs DA, Nussenblatt RB, Rosenbaum JT. Standardization of uveitis nomenclature for reporting clinical data. Results of the First International Workshop. *Am J Ophthalmol* 2005;140:509–16.
5 European Medicines Agency. EU regulatory workshop—ophthalmology—summary and report. Clinical development, scientific advice and paediatric investigation plans. *EMA*. 2012 http://www.ema.europa.eu/ docs/en_GB/document_library/Report/2012/09/ WC500131815.pdf
6 Ladas JG, Wheeler NC, Morhun PJ, *et al*. Laser flare-cell photometry: methodology and clinical applications. *Surv Ophthalmol* 2005;50:27–47.
7 Woreta F, Thorne JE, Jabs DA, *et al*. Risk factors for ocular complications and poor visual acuity at presentation among patients with uveitis associated with juvenile idiopathic arthritis. *Am J Ophthalmol* 2007;143:647–55.
8 Gregory AC, Kempen JH, Daniel E, *et al*. Risk factors for loss of visual acuity among patients with uveitis associated with juvenile idiopathic arthritis; the systemic immunosuppressive therapy for eye diseases study. *Ophthalmology* 2013;120:186–92.
9 Holland GN, Denove CS, Yu F. Chronic anterior uveitis in children: clinical characteristics and complications. *Am J Ophthalmol* 2009;147:667–78.
10 Thorne JE, Woreta F, Kedhar SR, *et al*. Juvenile idiopathic arthritis-assoacited uveitis: incidence of ocular complications and visual acuity loss. *Am J Ophthalmol* 2007;143:840–6.
11 Davis JL, Dacanay LM, Holland GN, *et al*. Laser flare photometry and complications of chronic uveitis in children. *Am J Ophthalmol* 2003;135:763–71.
12 Holland GN. A reconsideration of anterior chamber flare and its clinical relevance for children with chronic anterior uveitis. *Trans Am Ophthalmol Soc* 2007;105:344–64.
13 Lovell DJ, Ruperto N, Goodman S, *et al*. Adalimumab with or without methotrexate in juvenile rheumatoid arthritis. *N Engl J Med* 2008;359:810–20.
14 Brunner HI, Ruperto N, Zuber Z, *et al*. Efficacy and safety of tocilizumab in patients with polyarticular-course juvenile idiopathic arthritis: results from a phase 3, randomised, double-blind withdrawal trial. *Ann Rheum Dis* 2015;74:1110–7.
15 Sen ES, Morgan MJ, MacLeod R, *et al*. Cross sectional, qualitative thematic analysis of patient perspectives of disease impact in juvenile idiopathic arthritis-associated uveitis. *Pediatr Rheumatol Online J* 2017;15:58.
16 Heilinghaus A, Foeldvari I, Edelsten C. Proposed outcome measures for prospective clinical trials in juvenile idiopathic arthritis-associated uveitis: a consensus effort from the multinational interdisciplinary working group for uveitis in childhood. *Arthritis Care Res* 2012;64:1365–72.
17 Jaffe GJ, Dick AD, Brézin AP, *et al*. Adalimumab in patients with active noninfectious uveitis. *N Engl J Med Overseas Ed* 2016;375:932–43.
18 Nguyen QD, Merrill PT, Jaffe GJ, *et al*. Adalimumab for prevention of uveitic flare in patients with inactive non-infectious uveitis controlled by corticosteroids (VISUAL II): a multicentre, double masked, randomised, placebo-controlled phase 3 trial. *Lancet* 2016;388:1183–92.

Rheumatology 4.0: big data, wearables and diagnosis by computer

> We must dare to think 'unthinkable' thoughts. We must learn to explore all the options and possibilities that confront us in a complex and rapidly changing world. J. William Fulbright, US Politician. 9 April 1905–9 February 1995
>
> Nothing is unthinkable, nothing impossible to the balanced person, provided it comes out of the needs of life and is dedicated to life's further development. Lewis Mumford, US historian, sociologist, philosopher and literary critic. 19 October 1895–26 January 1990

Let's jump into the future, about 10 years from now. *Sandy Myers* (fictional name) is a 44-year-old female IT specialist. She is suffering from joint pain, morning stiffness, swollen finger joints and toes, as well as fatigue since 4 months. What should she do now? You would say, she should see rheumatologist as soon as possible and attend an early arthritis clinic. But will we have enough rheumatologists in 10 years, will this be common practice? Instead, she walks into the living room in the morning and addresses *Alessia* (fictional name), her cloud-based voice service machine. She tells *Alessia* about her health problems, and the machine takes a detailed history asking her several questions, which have been elaborated by a EULAR initiative (EULAR recommendations on core rheumatology questions in cloud-based voice service. Annals of the Rheumatic Diseases 2025): how can I help you?, where do you have your pain?, is it more pronounced in the morning?, do non-steroidals help?, do you feel like having an influenza? and some others. The answers are entered online into a structured patient history databank.

Sandy is now asked by Alessia to perform a self-examination that is helped by a large screen on her living room wall. Showing examples of diseased joints and with the help of animated pictures, Sandy examines her painful joints and records if they are swollen. This is aided by a little camera provided by the cloud-based system that also measures the circumferences of her finger joints and toes. All these findings are automatically entered into Sandy's health databank where many data are already available, most notably those of her 'Healthbit' wearable device. The activity tracker has recorded a reduced number of steps: from 8500 down to an average to 4700. She avoids stairs, but her cardiovascular system as constantly monitored by EKG, blood pressure and pulse rate is fine. There is also no fever.

Subsequently, Sandy goes to supermarket for shopping. However, prior to entering the store, there is a little booth that besides dry cleaning, shoe repairs and key cutting offers instant health services such as imaging diseased joints and blood-based biomarker analysis. Sandy enters this area, and a quick scan of the finger and wrist joints is performed using a novel laser-based scanning technique. Luckily, she is already equipped with a small implantable device underneath the skin of her forearm where special health data are stored, which need special data protection and she did not want to have in the cloud. Here, all her genomic data are stored thanks to a gift by her husband who donated her full genome sequencing as a gift for last Christmas. She puts her forearm in the reading device, and also some drops of blood are taken by a small lancet for further analysis in the machines in the basement of the supermarket run by robots.

These analyse the gene expression profile of the leucocyte subsets as well as the proteomic and immunomic profile. Macrophage activation is highly positive; there are no signs of infections, certain soluble factors indicative of an inflammatory joint disease are elevated, and IgA and IgM rheumatoid factors as well as ACPA are detected. There is also the expression of anti-Ro antibodies. The genomic profile is read out and reveals HLA shared epitope positivity as well as certain genes indicative of a reduced activity of enzymes involved in the metabolism of non-steroidal drugs.

So, which data are available to guide in Sandy's diagnosis? We have:

- Patient history.
- Joint status.
- Imaging data.
- Wearable device data.
- Genetic data.
- Gene expression data.
- Autoantibodies.

Based on the diagnostic algorithms, the computer calculates the diagnosis: rheumatoid arthritis with a probability of >99%. Who would disagree with this diagnosis? And interestingly, the patient has not been seen by a physician let alone a rheumatologist.

What will Sandy do now after she has received the diagnosis, of course with an empathic and comprehensive explanation by the computer, how well this disease can be treated? She asks the computer which therapeutic options are available, and it offers four treatment options to Sandy using detailed animated decision aids, carefully outlining the pros and cons of each therapy:

1. Biological A.
2. A tsDMARD.
3. Combination treatment with biological A plus a tsDMARD.
4. Biologic A, a tsDMARDs plus regulatory chimeric antigen receptor (CAR) T cells, a technology that had been developed in haemato-oncology and has now been introduced to treat autoimmune diseases.

Sandy opts for choice 4, since she feels that this combination—even though quite elaborate—offers the best chances of a cure. She is now finally referred to a 'Rheumatology 4.0 Unit' in a large university-based centre that will provide this treatment. Interestingly, Sandy's health insurance company requests to monitor her adherence to the quite expensive tsDMARD using digital monitoring with an ingestible event marker (IEM), a capsule that on entering the stomach sends a signal to a central remote tracking system via a patch and her smartphone. In addition, T cell cultures derived from her blood via leukapheresis are expanded to yield regulatory CAR T cells. A 3-monthly evaluation of the cellular gene expression profile is performed, and after 6 months, treatment is changed to therapy with CAR T cells due to a favourable gene expression profile. After 1 year, there is a complete drug-free remission without any further deterioration of imaging scans and no further treatment.

Is this case pure science fiction or will this scenario be possible in the future? Let's now do a reality check on what already is available now.

Many people believe that cloud-based voice service machines may replace our conventional means of entering data into a computer, pretty much as we already saw in the *Star Trek* movies many years ago. Classical keyboards and stand-alone computers will be nearly extinct in the future or be used only for dedicated tasks. Of course, a sophisticated program will be necessary to ask the right questions, analyse them and put them into context of the other data. Self-examination programs using pictures of diseased joints and skin are already available and used in certain early arthritis projects.[1] Mobile devices will potentially extend the reach of specialists outside of the clinic setting. A forecast is that 6.3 billion smartphone subscriptions will exist by the year

2021 and can therefore potentially provide inexpensive universal access to diagnostic care.[2] There will also be a tremendous progress in wearable technology[3] that already now records activity (steps, stairs and calories used) and cardiovascular data and will be extended to the constant monitoring of serum parameters such as glucose and potentially even C reactive protein. These will also help monitoring drug adherence using digital monitoring with an 'IEM'.[4]

Implantable devices to store data are available as well[5] and whole genome typing will be quite inexpensive in the future. Instant scanning methods to assess inflammation already document involved joints, currently using fluorescence optical imaging,[6] which is invasive, but non-invasive methods will be developed. Already now (however by rather complicated methods) gene expression analysis is possible and can be compared with thousands of databases already publicly available.[7] These can discriminate between states of viral, bacterial or other inflammations. Proteomics and autoantibody analysis can be done with sophisticated and rapid techniques. Thus, many of these data can already be recorded today, and the major step forward will then be the process, which is called 'cognitive computing' using elements of artificial intelligence as well as 'deep' and machine ('self') learning and which assesses structured (ie, laboratory, imaging and activity) and unstructured data (ie, text). Interestingly, machine learning had been developed to excel in games such as checkers and chess and was then transferred to several completely distinct disciplines. In medical terms, there is a very good explanation of machine learning by Obermeyer and Emanuel[8]:

> Machine learning… approaches problems as a doctor progressing through residency might: by learning rules from data. Starting with patient-level observations, algorithms sift through vast numbers of variables, looking for combinations that reliably predict outcomes. In one sense, this process is similar to that of traditional regression models: there are outcomes, covariates, and statistical functions linking the two. But where machine learning shines is in handling enormous numbers of predictors—sometimes, remarkably, more predictors than observations—and combining them in nonlinear and highly interactive ways. This capacity allows us to use new kinds of data, whose sheer volume or complexity would previously have made analyzing them unimaginable.

Recently, in dermatology, 'deep convolutional neural networks' were reported to achieve performance similar to all tested experts demonstrating an artificial intelligence capable of classifying skin cancer with a level of competence comparable to 'human' dermatologists using mobile devices.[2]

Going back to Sandy's treatment, we now have multitude of biologicals, tsDMARDs and also optimised strategies[9 10] how to use them wisely, especially in very early disease such is the case with Sandy.[11] Of course, transferring the breath-taking CAR technology from haemato-oncology to autoimmune diseases may still be science fiction, but initial approaches are already made.[12]

You may finally ask what the role of the physician may be in 10 years from now considering the scenario above. Certainly, many diagnostic algorithms will be performed by computer-aided systems, but the physician will still guide the patient through the various options, especially if it comes to difficult therapeutic decisions where a personal experience will be of great importance, potentially involving a multidisciplinary team. Of course, the actual treatment and management 'at the front line' will need doctors and nurses, particularly in emergency medicine and in a ward setting.

In conclusion, many of the approaches outlined above will use 'Big Data', which is based on the expectation that computerised algorithms performing calculation, data processing and automated reasoning tasks may extract new knowledge from otherwise unmanageable vast data sets.[13] In rheumatology, high throughput technologies in molecular research already generated big data in rheumatology some 15 years ago. These included 'omics' technologies, such as genomics, transcriptomics and cytomics. In the future, functional analysis and interpretation will require adaptation of existing and the development of new software tools frequently based on machine learning. Structuring and evaluating the biological (medical) context will be essential and will not just be a mathematical problem. More and more extensive recording of disease characteristics and molecular processes in individual patients will generate personal big data and will require new strategies for management to develop data-driven individualised concepts resulting in a better diagnosis and treatment. Regarding our profession, this will also require new approaches in education and professional competence. Obermeyer and Lee have again very well addressed this, and thus I end with a quote[14]:

> There is little doubt that algorithms will transform the thinking underlying medicine. The only question is whether this transformation will be driven by forces from within or outside the field. If medicine wishes to stay in control of its own future, physicians will not only have to embrace algorithms, they will also have to excel at developing and evaluating them, bringing machine learning methods into the medical domain.

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REFERENCES

1 Schnelle Hilfe bei Rheuma. https://www.rheport.de/Start.aspx (accessed 02 May 2018).
2 Esteva A, Kuprel B, Novoa RA, *et al*. Dermatologist-level classification of skin cancer with deep neural networks. *Nature* 2017;542:115–8.

3 Pevnick JM, Birkeland K, Zimmer R, *et al*. Wearable technology for cardiology: an update and framework for the future. *Trends Cardiovasc Med* 2018;28:144–50.
4 Thasso. Abilify MyCite, the first digital pill. Where does all that silicone go? http://thasso.com/abilify-mycite-the-first-digital-pill-where-does-all-that-silicon-go (accessed 02 May 2018).
5 New range of flexible implantable devices to launch later this month. https://www.xda-developers.com/140883-2 (accessed 02 May 2018).
6 Krohn M, Ohrndorf S, Werner SG, *et al*. Near-infrared fluorescence optical imaging in early rheumatoid arthritis: a comparison to magnetic resonance imaging and ultrasonography. *J Rheumatol* 2015;42:1112–8.
7 BioRetis. http://www.bioretis.com/ (accessed 02 May 2018)
8 Obermeyer Z, Emanuel EJ. Predicting the future - big data, machine learning, and clinical medicine. *N Engl J Med* 2016;375:1216–9.
9 Burmester GR, Pope JE. Novel treatment strategies in rheumatoid arthritis. *Lancet* 2017;389:2338–48.
10 Smolen JS, Breedveld FC, Burmester GR, *et al*. Treating rheumatoid arthritis to target: 2014 update of the recommendations of an international task force. *Ann Rheum Dis* 2016;75:3–15.
11 Bijlsma JWJ, Welsing PMJ, Woodworth TG, *et al*. Early rheumatoid arthritis treated with tocilizumab, methotrexate, or their combination (U-Act-Early): a multicentre, randomised, double-blind, double-dummy, strategy trial. *Lancet* 2016;388:343–55.
12 Biswas M, Kumar SRP, Terhorst C, *et al*. Gene therapy with regulatory T Cells: a beneficial alliance. *Front Immunol* 2018;9:554.
13 Bonin-Andresen M, Smiljanovic B, Stuhlmüller B, *et al*. [Relevance of big data for molecular diagnostics]. *Z Rheumatol* 2018;77:195–202.
14 Obermeyer Z, Lee TH. Lost in thought - the limits of the human mind and the future of medicine. *N Engl J Med* 2017;377:1209–11.

Defining refractory rheumatoid arthritis

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ABSTRACT

While biologic disease-modifying antirheumatic drugs (bDMARDs) have transformed outcomes of people with rheumatoid arthritis (RA), a proportion of patients are refractory to multiple bDMARDs. Definitions of refractory RA thus far have been arbitrary, and outcome data and impact of such cohorts remain limited. Extrapolation from randomised controlled trial and some real-life data suggest approximately 20% progress onto a third bDMARD with a more modest proportion failing additional bDMARDs. This viewpoint discusses an opinion of refractory RA disease and proposes key principles to accurately identify refractory cohorts. These include demonstrating presence of persistent inflammation despite multiple therapies and acknowledging development of antidrug antibody. Potential basis of refractory disease is summarised, and suggestions for an initial approach in the future evaluation of refractory disease are offered. Specific investigation of refractory RA disease is necessary to inform the clinical need and provide a basis for robust investigation of underlying mechanisms.

BACKGROUND

While targeted therapies have transformed the management of rheumatoid arthritis (RA), refractory disease to multiple biologic disease-modifying antirheumatic drugs (bDMARDs) presents a significant clinical challenge. Extrapolating from randomised controlled trial (RCT) data (with low hurdle response endpoints), approximately 40% failure to a first bDMARD[1] and another 40% on a second bDMARD,[2–4] implies almost 20% progress to a third bDMARD. Disease progression and impact in such a multi-bDMARD cohort is not clear, a point highlighted by historical studies illustrating lack of structural progression in tumour necrosis factor inhibitor (TNFi)-treated cohorts.[5] Our understanding of refractory RA disease thus remains limited.

This viewpoint focuses on multi-bDMARD inefficacy and proposes an approach to identify true (intrinsic) refractory RA disease (distinct from antidrug antibody (ADA)-mediated non-response) and possible underlying mechanisms. In the absence of a dedicated evidence base to refer to, this viewpoint reflects an opinion to highlight the need for and inform future initiatives.

SETTING THE SCENE

Refractory *disease* is broadly assumed to imply resistance/refractoriness of multiple agents, more than might be considered 'normal' or 'reasonable' for the specific disease. Prior to the introduction of bDMARDs, refractory RA denoted multiple conventional synthetic DMARD failure,[6] although methotrexate (MTX)-inadequate response (IR) RCTs have historically not mandated failure to optimal dose MTX. With escalation to TNFi (as the first available bDMARD class), a subsequent (multiple) TNFi-failure cohort emerged. We now observe a cohort that has failed several bDMARD classes. The only registry data in this field report 5% failing at least third bDMARD class due to inefficacy and/or toxicity.[7] RCTs typically demonstrate 50%–30%–10% ACR 20, 50 and 70 responses, respectively, in TNFi-IR studies.[2–4] Thus, the vast proportion (over 70%) of patients on a second bDMARD *class* fail to actually derive a meaningful clinical response.

IDENTIFYING REFRACTORY RA DISEASE: SHOULD NECESSARILY MEAN REFRACTORY INFLAMMATION

Targeted agents are designed a priori to interfere with key mediators of inflammation and thus suppress synovial inflammation, the primary site of pathology and driver of joint damage.[8] Thus, the assessment of an individual with RA refractory to a single and/or multiple DMARDs should necessarily mean presence of persistent (proven) inflammation, be it local synovitis and/or systemic inflammation.

Recognising discordance between measured disease activity and pathology

While clinical tools such as DAS28 are well-established validated surrogate measures of synovitis and employed for response assessment,[9] limitations in this are well-recognised. Discordance is observed between clinically judged disease activity and validated clinical tools, the latter sometimes disproportionately driven by the more subjective components.[10 11] Secondary damage and osteoarthritis associated with increasing disease duration as well as (poorly understood) chronic pain states are recognised to distort measured active disease state and thus could reasonably drive apparent 'refractory' drug profiles. If indicated, assessment of inflammation can be strengthened by imaging, with presence of power Doppler ultrasound, a credible measure that has been linked to damage.[12]

Identifying pharmacokinetic drivers and intrinsic refractory disease

Non-response is typically categorised as primary or secondary non-response based on whether an initial (usually defined as week 12–16) response to an intervention is observed or not.[13–15] Incorrect targeting (ie, a mismatch between key disease mediator(s) and drug target) is a principal concept in the investigation of drug non-response (discussed later). Primary non-response has been presumed to be indicative of this. This suggestion of intrinsic disease resistance contrasts with pharmacokinetic factors in which drug is on target, but ADA-drug

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immune complexes lead to abrogation of pharmacological activity of the drug and/or enhanced drug clearance. This phenomenon is clinically relevant; it can be measured and potentially circumvented using alternative within-class bDMARD.[15 16] ADA, however, tend to be considered in the context of secondary resistance but could conceivably develop in the earliest stages of treatment exposure.[16 17] The conventional approach of using timing of non-response as a surrogate for incorrect targeting or ADA thus remains an assumption. Instead, an unbiased approach to demonstrate the basis of non-response would be more accurate clinically meaningful.

MECHANISMS UNDERLYING REFRACTORY RA DISEASE

No studies have specifically investigated the biological basis of *multi*-bDMARD refractoriness. Efficacy of non-TNF-targeted therapies in TNFi refractory cohorts and post-hoc analyses[4] is used to support the assertion of incorrect drug targeting (or pathological accuracy) as the basis for individual refractory drug response. Comparative trials such as the 'ROC' trial[18] in which non-TNF bDMARD class was superior to alternative TNFi in individuals with first TNFi lack of efficacy further suggests this interpretation (although limited by the non-TNFi arm comprising three different bDMARD classes). TNFi to TNFi switch however has been demonstrated to be effective,[19] and the theory of incorrect targeting also does not necessarily play out in clinical practice where we often see strikingly opposite primary responses with within-class (TNFi) cycling.[20 21]

Furthermore, experimental investigations have not yet validated this concept.[22] Within the elegantly described biological paradigm[23] underpinned by clinical observation of a cytokine network that is host to key druggable nodes (such as TNF, IL-6 and GM-CSF), the inter-relationship of such cytokine nodes (does response to TNFi implicitly indicate TNF-driven disease not amenable to IL-6 targeting?), whether 'adaptation' to alternative key nodes or mechanisms may occur, and whether and how multi-bDMARD refractory RA fits in this concept, is unclear. Figure 1 further summarises the possible pathophysiological basis of refractory disease (a detailed appraisal of which is outside the scope of this viewpoint).

RESEARCH AGENDA

As a starting point, future research in multi-drug refractory RA disease requires consistent definitions and criteria.

Overarching definition of refractory RA disease

Similar to that employed in cancer therapy, refractory RA disease could on a generic level be defined as:

- resistance to multiple therapeutic drugs with different structures and mechanisms of action.

Following optimal dose MTX inefficacy, the number of prior bDMARD an individual's RA disease needs to be refractory to before classified as multi-drug refractory disease is not implicitly clear. Multiple within-class bDMARD resistance (as with TNFi cycling) would not seem compatible with refractory RA *disease*. With current bDMARD classes comprising two broad mechanisms (anticytokine and cell-targeted agents), one could in the first instance suggest the following:

- failure of at least one anticytokine (TNF and/or IL-6 directed) and one cell-targeted (B cell depletion and/or T cell costimulation blockade) bDMARD.

The advent of Janus kinase inhibitors (JAKi), the first targeted synthetic molecule, adds another layer of complexity to the above definition that in time may incorporate failure to a targeted synthetic therapy.

Evaluating ADA and non-inflammatory drivers of refractory RA

Identifying ADA and non-inflammatory pathologies in clinical studies are necessary to be able to classify refractory response. Central to this is confirming persistent inflammation (synovitis and/or systemic), distinct from solely clinically relevant biomechanical and degenerative drivers, so that we do not only include a surrogate for longer disease duration and damage.[24] Ultrasound imaging and presence of power Doppler is appropriate if/

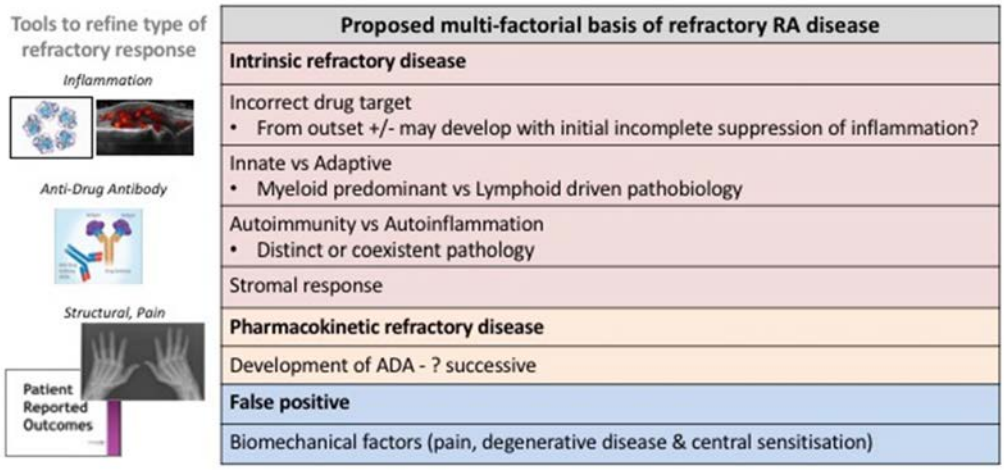

Figure 1 Refractory disease that is not as a consequence of ADA and/or biomechanical factors may represent disease subgroups with distinct immunopathological drivers. Different cytokine/cell pathway targets and associations between synovial tissue pathobiological subtype, associated genes and response to bDMARD have been suggested.[28] The role of both innate and adaptive drivers of disease, and observation of autoinflammatory phenotype with/without coexistent typical autoantibody-mediated disease[29] provides an additional basis for refractory subgroups. The relevance of the stromal response and effect of FLS-derived cytokines in driving persistence of synovitis in refractory RA disease might also be particularly relevant.[30] ADA, antidrug antibody; bDMARD, biologic disease-modifying antirheumatic drug; FLS, fibroblast-like synoviocytes; RA, rheumatoid arthritis.

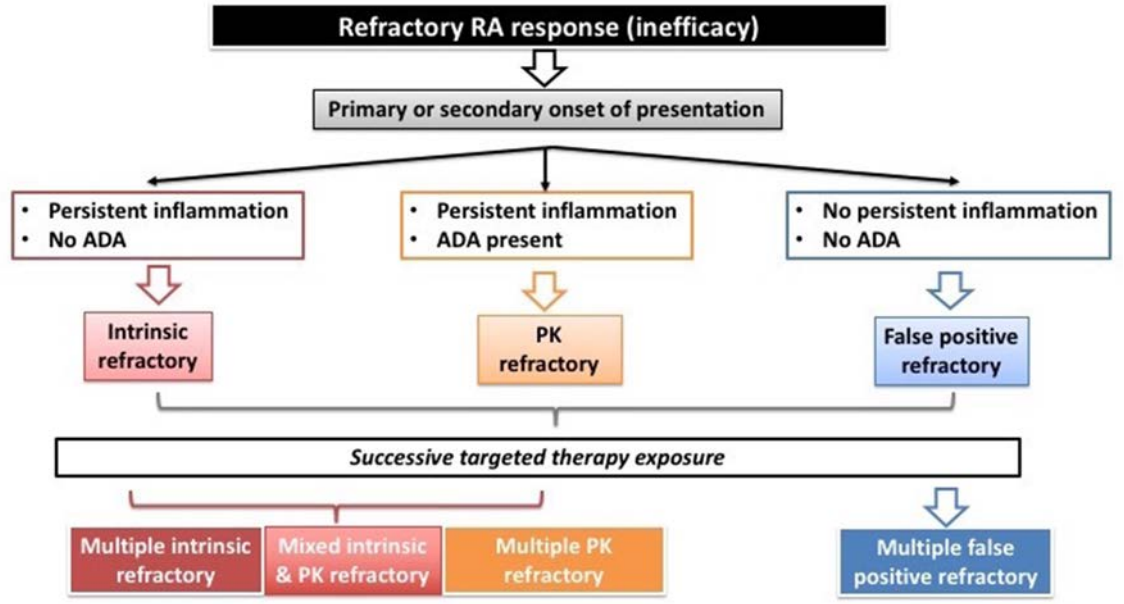

Figure 2 Categorisation of individual and successive refractory drug response.* *Disease activity status may be verified with additional use of imaging such as ultrasound to confirm presence/absence of synovial inflammation. ADA, antidrug antibody; PK, pharmacokinetic; RA, rheumatoid arthritis.

when clinical assessment is not clear.[25] The presence or absence of ADA can provide further clarification such that refractory response can be stratified into the following groups (illustrated in figure 2 and also applied to categorisation of successive refractory drug outcomes):

- intrinsic refractory: persistent inflammation, no ADA (with/without secondary damage)
- pharmacokinetic refractory: persistent inflammation with ADA
- false refractory: absence of inflammation; other (biomechanical±degenerative) drivers.

Coexistence of incorrect targeting with ADA is theoretically possible, but perhaps somewhat academic as the former could not be overcome without bDMARD class switch.

Minimum clinical target to determine refractory disease

Building on the treat to target principles of RA management,[26] a minimum target tailored to the individual should be set that if not achieved would determine refractory disease state. Moderate disease activity (as measured by a validated scoring tool) may be the most appropriate in a large proportion (taking into account prevalent populations that may have accrued extensive secondary consequences of disease). For newly diagnosed patients, achievement of clinical target within 6 months of commencing conventional synthetic DMARD is advocated. Applying similar principles, intrinsic failure of a minimum of two classes of bDMARD would be reached within 18–24 months, providing an initial indication of time course in the development of multi-bDMARD refractory patients.

Investigating the biological relationship between synovial inflammation and pain

Observations from the more recent RCTs of targeted synthetic JAKis suggest differential pain modification to bDMARDs,[27] implying the presence of distinct biological drivers of pain that are independent to the peripheral sensitisation of synovitis. Such data raise interesting hypotheses on the role of JAKi in pain pathways and highlight the importance of not restricting evaluation of refractory disease to just that associated with synovial inflammation.

CONCLUDING REMARKS

This viewpoint highlights the knowledge gap in the identification and understanding of refractory RA disease. In the absence of well-phenotyped studies and a systematic approach to evaluating refractory RA disease, the true extent, impact and underlying basis remains unclear. Disease duration and damage that are associated with a suboptimal patient response profile blur the precision with which we may be sequencing therapies and estimating the size of the problem. Suboptimal targeting of disease from the outset may also be implicated in the development of refractory pathology. The heterogeneous nature of RA and pharmacokinetic drivers of drug response both likely play a role in leading to this clinical landscape. Continued drug development pipeline, therefore, remains important to offer ongoing opportunities.

Correction notice This article has been corrected since it published Online First. Figures 1 and 2 have been corrected.

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REFERENCES

1 van der Heijde D, Klareskog L, Rodriguez-Valverde V, *et al*. Comparison of etanercept and methotrexate, alone and combined, in the treatment of rheumatoid arthritis:

two-year clinical and radiographic results from the TEMPO study, a double-blind, randomized trial. *Arthritis Rheum* 2006;54:1063–74.
2 Genovese MC, Becker JC, Schiff M, *et al*. Abatacept for rheumatoid arthritis refractory to tumor necrosis factor alpha inhibition. *N Engl J Med* 2005;353:1114–23.
3 Cohen SB, Emery P, Greenwald MW, *et al*. Rituximab for rheumatoid arthritis refractory to anti-tumor necrosis factor therapy: Results of a multicenter, randomized, double-blind, placebo-controlled, phase III trial evaluating primary efficacy and safety at twenty-four weeks. *Arthritis Rheum* 2006;54:2793–806.
4 Emery P, Keystone E, Tony HP, *et al*. IL-6 receptor inhibition with tocilizumab improves treatment outcomes in patients with rheumatoid arthritis refractory to anti-tumour necrosis factor biologicals: results from a 24-week multicentre randomised placebo-controlled trial. *Ann Rheum Dis* 2008;67:1516–23.
5 Maini RN, Breedveld FC, Kalden JR, *et al*. Sustained improvement over two years in physical function, structural damage, and signs and symptoms among patients with rheumatoid arthritis treated with infliximab and methotrexate. *Arthritis Rheum* 2004;50:1051–65.
6 Bingham SJ, Buch MH, Tennant A, *et al*. The impact of escalating conventional therapy in rheumatoid arthritis patients referred for anti-tumour necrosis factor-alpha therapy. *Rheumatology* 2004;43:364–8.
7 Kearsley-Fleet L, De Cock D, Watson K, *et al*. Refractory disease in rheumatoid arthritis: results from the british society of rheumatology biologics register for rheumatoid arthritis. *Arthritis Rheumatol* 2017;69.
8 McGonagle D, Gibbon W, Emery P. Classification of inflammatory arthritis by enthesitis. *Lancet* 1998;352:1137–40.
9 Fransen J, van Riel PL. The Disease Activity Score and the EULAR response criteria. *Clin Exp Rheumatol* 2005;23:S93–9.
10 Ranzolin A, Brenol JC, Bredemeier M, *et al*. Association of concomitant fibromyalgia with worse disease activity score in 28 joints, health assessment questionnaire, and short form 36 scores in patients with rheumatoid arthritis. *Arthritis Rheum* 2009;61:794–800.
11 Lee YC, Frits ML, Iannaccone CK, *et al*. Subgrouping of patients with rheumatoid arthritis based on pain, fatigue, inflammation, and psychosocial factors. *Arthritis Rheumatol* 2014;66:2006–14.
12 Brown AK, Conaghan PG, Karim Z, *et al*. An explanation for the apparent dissociation between clinical remission and continued structural deterioration in rheumatoid arthritis. *Arthritis Rheum* 2008;58:2958–67.
13 Finckh A, Simard JF, Gabay C, *et al*. Evidence for differential acquired drug resistance to anti-tumour necrosis factor agents in rheumatoid arthritis. *Ann Rheum Dis* 2006;65:746–52.
14 Buch MH, Bingham SJ, Bejarano V, *et al*. Therapy of patients with rheumatoid arthritis: outcome of infliximab failures switched to etanercept. *Arthritis Rheum* 2007;57:448–53.
15 Schiff MH, von Kempis J, Goldblum R, *et al*. Rheumatoid arthritis secondary non-responders to TNF can attain an efficacious and safe response by switching to certolizumab pegol: a phase IV, randomised, multicentre, double-blind, 12-week study, followed by a 12-week open-label phase. *Ann Rheum Dis* 2014;73:2174–7.
16 Garcês S, Antunes M, Benito-Garcia E, *et al*. A preliminary algorithm introducing immunogenicity assessment in the management of patients with RA receiving tumour necrosis factor inhibitor therapies. *Ann Rheum Dis* 2014;73:1138–43.
17 Jani M, Chinoy H, Warren RB, *et al*. Clinical utility of random anti-tumor necrosis factor drug-level testing and measurement of antidrug antibodies on the long-term treatment response in rheumatoid arthritis. *Arthritis Rheumatol* 2015;67:2011–9.
18 Gottenberg JE, Brocq O, Perdriger A, *et al*. Non-TNF-targeted biologic vs a second anti-tnf drug to treat rheumatoid arthritis in patients with insufficient response to a first anti-tnf drug: a randomized clinical trial. *JAMA* 2016;316:1172–9.
19 Smolen JS, Kay J, Doyle MK, *et al*. Golimumab in patients with active rheumatoid arthritis after treatment with tumour necrosis factor alpha inhibitors (GO-AFTER study): a multicentre, randomised, double-blind, placebo-controlled, phase III trial. *Lancet* 2009;374:210–21.
20 Buch MH, Conaghan PG, Quinn MA, *et al*. True infliximab resistance in rheumatoid arthritis: a role for lymphotoxin alpha? *Ann Rheum Dis* 2004;63:1344–6.
21 Buch MH, Seto Y, Bingham SJ, *et al*. C-reactive protein as a predictor of infliximab treatment outcome in patients with rheumatoid arthritis: defining subtypes of nonresponse and subsequent response to etanercept. *Arthritis Rheum* 2005;52:42–8.
22 Romão VC, Vital EM, Fonseca JE, *et al*. Right drug, right patient, right time: aspiration or future promise for biologics in rheumatoid arthritis? *Arthritis Res Ther* 2017;19:1–13.
23 Schett G, Elewaut D, McInnes IB, *et al*. How cytokine networks fuel inflammation: toward a cytokine-based disease taxonomy. *Nat Med* 2013;19:822–4.
24 Aletaha D, Smolen J, Ward MM. Measuring function in rheumatoid arthritis: Identifying reversible and irreversible components. *Arthritis Rheum* 2006;54:2784–92.
25 D'Agostino MA, Terslev L, Wakefield R, *et al*. Novel algorithms for the pragmatic use of ultrasound in the management of patients with rheumatoid arthritis: from diagnosis to remission. *Ann Rheum Dis* 2016;75:1902–8.
26 Smolen JS, Aletaha D, Bijlsma JWJ, *et al*. Treating rheumatoid arthritis to target: recommendations of an international task force. *Ann Rheum Dis* 2010;69:631–7.
27 Taylor PC, Keystone EC, van der Heijde D, *et al*. Baricitinib versus placebo or adalimumab in rheumatoid arthritis. *N Engl J Med* 2017;376:652–62.
28 Townsend MJ. Molecular and cellular heterogeneity in the rheumatoid arthritis synovium: clinical correlates of synovitis. *Best Pract Res Clin Rheumatol* 2014;28:539–49.
29 Savic S, Mistry A, Wilson AG, *et al*. Autoimmune-autoinflammatory rheumatoid arthritis overlaps: a rare but potentially important subgroup of diseases. *RMD Open* 2017;3:e000550–7.
30 Filer A. The fibroblast as a therapeutic target in rheumatoid arthritis. *Curr Opin Pharmacol* 2013;13:413–9.

OPEN ACCESS

CONCISE REPORT

Exposure to passive smoking and rheumatoid arthritis risk: results from the Swedish EIRA study

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ABSTRACT

Introduction Smoking has consistently been associated with increased risk of developing rheumatoid arthritis (RA). The aim of this study was to estimate the influence of passive smoking on the risk of developing anti-cyclic citrullinated peptide antibodies (ACPA)-positive and ACPA-negative RA.

Methods A population-based case–control study using incident cases of RA was performed in Sweden, and the study population in this report was restricted to include never-smokers (589 cases, 1764 controls). The incidence of RA among never-smokers who had been exposed to passive smoking was compared with that of never-smokers who had never been exposed, by calculating the OR with a 95% CI employing logistic regression.

Results No association was observed between exposure to passive smoking and RA risk (OR 1.0, 95% CI 0.8 to 1.2 for ACPA-positive RA, and OR 0.9, 95% CI 0.7 to 1.2, for ACPA-negative RA). No suggestion of a trend between duration of passive smoking and RA risk was observed.

Discussions No association was observed between exposure to passive smoking and RA risk, which may be explained by a threshold below which no association between smoke exposure and RA occurs.

INTRODUCTION

Rheumatoid arthritis (RA) is systemic inflammatory disease characterised by progressive joint destruction and autoantibody formation. Based on serological features, RA can be divided into anti-cyclic citrullinated peptide antibodies (ACPA)-positive and ACPA-negative subsets.[1] Disease susceptibility is determined by a complex interplay between genetic and environmental factors, and both retrospective and prospective studies have demonstrated that smoking is one of the major environmental factors in RA development.[2–5] Smoking has been observed to induce citrullination of peptide antigens in the lungs[6] and has been reported to be an important factor for the development of RA in the ACPA-positive subset.[7]

No studies have been performed investigating the effect of environmental tobacco smoke on RA risk. However, maternal smoking during pregnancy has been reported to increase the risk of inflammatory polyarthropathies and juvenile RA in female offspring.[8] The effect of environmental tobacco smoke on disease activity in RA has been investigated in a multicentre longitudinal observational study of patients with RA, and no impact of passive smoking on disease activity was observed among never-smoking patients with RA.[9] Using a large Swedish population-based case–control study we thus aimed to examine whether exposure to passive smoking influences the risk of developing ACPA-positive and ACPA-negative RA.

METHODS

Study design and study subjects

This report was based on data from the ongoing project Epidemiological Investigation of Rheumatoid Arthritis (EIRA) which is a population-based case–control study comprising the population aged 18–70 years in the middle and southern parts of Sweden. All hospital-based and most privately run rheumatology units participated in recruiting incident cases to the study. All cases fulfilled the American College of Rheumatology 1987 criteria. For each case, two controls were randomly selected from the national population register, matched by age, gender and residential area. A more detailed description of the study design can be found elsewhere.[10]

During the study period October 2005 to September 2014, completed questionnaires were obtained from 1652 cases and 3553 controls, the response proportion being 92% for the cases and 75% for the controls.

Anti-cyclic citrullinated peptide antibodies

ACPA status among cases was analysed using Immunoscan-RA Mark2 ELISA test (anti-CCP2 test). An antibody level exceeding 25 AU/mL was regarded as ACPA positivity.

Data collection

Information regarding lifestyle factors and different exposures was collected using a standardised questionnaire. Information on smoking was obtained by asking about current and previous smoking habits, and information on exposure to passive smoking was obtained by asking if the subject had been daily exposed to passive smoking at home or at work, and if so, during what period or periods in life.

For each case, the time of the initial appearance of RA symptoms was used as an estimate of the disease onset, and the year in which this occurred was defined as the index year. The corresponding controls were given the same index year. Information regarding smoking and exposure to passive smoking was considered prior to or during the index year in the cases and during the same period of time in the corresponding controls.

All ever-smokers were excluded (1063 cases and 1789 controls). Never-smokers who reported exposure to passive smoking prior to index were

defined as exposed whereas those who reported that they had never been exposed to passive smoking were defined as never exposed. Exposed subjects were also divided into groups based on whether the exposure occurred within 10 years prior to index or earlier in life. In order to analyse the influence of duration of exposure to passive smoking on the risk of developing the disease, we categorised the exposed subjects into groups based on the duration of exposure prior to index.

Statistical analysis

Among never-smokers, subjects exposed to passive smoking were compared with those that reported never having been exposed to passive smoking with regard to occurrence of RA, by calculating ORs with 95% CIs employing logistic regression. Trend test for a dose–response relationship regarding duration of passive smoking and risk of both subsets of RA was performed by using a continuous variable for duration of passive smoking (years) in a logistic regression model.

Both matched and unmatched analyses were carried out. The results from the unmatched analyses were in close agreement with those from the matched analyses but had a higher degree of precision due to a substantial loss of cases and controls in the matched analyses.

All analyses were adjusted for age, gender, residential area and ancestry. Assessment of ancestry was based on whether the subject was born in Scandinavia or not, and whether either of the subject's parents had immigrated to Scandinavia. A subject who was born in Scandinavia, whose parents had not immigrated, was classified as Scandinavian. Adjustments were also made for educational level (university degree or not), alcohol consumption (number of drinks per week at study inclusion) and body mass index at inclusion in the study (more or less than 25 kg/m^2). However, these factors had a minor influence on the results and were not retained in the final analyses. All analyses were conducted using SAS V.9.2.

RESULTS

Our analyses of passive smoking and RA risk among never-smokers included 589 cases and 1764 controls. Overall, the proportion that had been exposed to passive smoking prior to the index year was 47% among ACPA-positive cases, 48% among ACPA-negative cases and 50% among controls. Characteristics of cases and controls are presented in table 1.

No association was observed between exposure to passive smoking and risk of ACPA-positive or ACPA-negative RA, regardless if the exposure took place within 10 years prior to index or earlier in life. Compared with those who had never been exposed to passive smoking, the OR was 1.0 (95% CI 0.7 to 1.2) for ACPA-positive RA and 0.9 (95% CI 0.7 to 1.2) for ACPA-negative RA among those ever exposed to passive smoking (table 2). The results from the matched analysis are presented in online supplementary table 1. There were no significant age-related or gender-related differences. There was no suggestion of a trend between duration of passive smoking and RA risk. Long-term exposure to passive smoking for 20 years or longer was not significantly associated with increased disease susceptibility (table 3).

Table 1 Characteristics of cases and controls

	ACPA-positive cases	ACPA-negative cases	Controls
Exposed to passive smoking, n (%)	180 (47)	98 (48)	876 (50)
Women, n (%)	301 (78)	137 (67)	1293 (73)
Men, n (%)	84 (22)	67 (33)	471 (27)
Scandinavian, n (%)	333 (86)	185 (91)	1533 (87)
Mean age at disease onset (SD)	52.8 (16.0)	47.8 (15.3)	
Disease duration since first symptom (SD)	0.8 (1.1)	0.9 (1.2)	
Total, n	385	204	1764

ACPA, anti-cyclic citrullinated peptide antibodies.

Table 2 OR with 95% CI of developing rheumatoid arthritis (RA) for subjects exposed to passive smoking compared with those who have never been exposed

Exposure to passive smoking	ACPA-positive RA		ACPA-negative RA	
	ca/co	OR (95% CI)*	ca/co	OR (95% CI)*
Never	205/888	1.0 (reference)	106/888	1.0 (reference)
Ever	180/876	1.0 (0.8 to 1.2)	98/876	0.9 (0.7 to 1.2)
Within 10 years prior to index	45/170	1.1 (0.8 to 1.7)	15/170	0.7 (0.4 to 1.3)
Before 10 years prior to index	135/706	0.9 (0.7 to 1.2)	83/706	1.0 (0.7 to 1.3)

All subjects were never-smokers.
*Adjusted for age, gender, residential area and ancestry.
ACPA, anti-cyclic citrullinated peptide antibodies; ca/co, number of exposed cases and controls.

DISCUSSION

According to our observations among never-smokers, exposure to passive smoking did not increase the risk of ACPA-positive or ACPA-negative RA. The present result of a lack of association between passive smoking and risk for RA may be due to the previously described threshold for exposure to smoke, where no association between risk for RA and active smoking was seen when the accumulated amount of smoking was low enough.[10] However, we only had information on duration but not intensity of exposure to passive smoking and we were therefore unable to calculate the cumulative dose of passively inhaled smoke in order to study a dose–response correlation.[11] Although exposure to passive smoking does not seem to be a risk factor for RA, we cannot rule out that extensive exposure to passive smoking could affect disease risk.

Our study was designed as a case–control study with incident cases, and information regarding smoking habits and exposure to passive smoking was collected retrospectively. Recall bias was minimised by using incident cases of RA. The mean duration

Table 3 OR with 95% CI of developing rheumatoid arthritis (RA) for subjects exposed to passive smoking compared with those who have never been exposed, by duration of exposure

Duration of exposure to passive smoking (years)	ACPA-positive RA		ACPA-negative RA	
	ca/co	OR (95% CI)*	ca/co	OR (95% CI)*
0	205/897	1.0 (reference)	108/897	1.0 (reference)
1–9	52/221	1.0 (0.7–1.5)	18/221	0.7 (0.4–1.2)
10–19	66/319	1.0 (0.7–1.3)	33/319	0.9 (0.6–1.3)
20-	616/325	0.9 (0.7–1.3)	45/325	1.1 (0.7–1.6)
P values for trend		0.8		0.8

All subjects were never-smokers.
*Adjusted for age, gender, residential area and ancestry.
ACPA, anti-cyclic citrullinated peptide antibodies; ca/co, number of exposed cases and control.

from the disease onset to inclusion in the study was <1 year in both subgroups. We took great effort to obtain information on lifestyle factors and environmental exposures in an identical way for the cases and the controls. Furthermore, the questionnaire contained a wide range of questions regarding many potential environmental risk factors and no section in the questionnaire was given prime focus.

A potential selection bias may arise when recruiting cases and controls. The proportion of respondents with regard to participation in EIRA was 92% for cases and 75% for controls. Since the structure of the Swedish public healthcare system provides equal access to medical services for all Swedish citizens, almost all cases of RA are referred to public rheumatology units and it is not likely that the few unidentified cases would cause a substantial bias in our calculations. Selection bias among controls is likely to be modest since the prevalence of smoking among controls, seen as an indicator of lifestyle, was in line with that of the general population at equivalent ages.[12]

When observing no association between exposure and disease, as in our study, it is of interest to know what strengths of association the study had a reasonably power to detect. Comparing ever exposed to passive smoking to never exposed, our study had the power (≥80) to identify an OR of 1.33 for ACPA-positive RA and 1.45 for ACPA-negative RA.

In summary, in this population-based case–control study of RA, no association was observed between exposure to passive smoking and risk of ACPA-positive or ACPA-negative RA among never-smokers. Our finding may be explained by a threshold below which no association between smoke exposure and RA occurs.

Contributors Conception and design of the study, and acquisition of data: LA and LK. Analysis of data and drafting of the manuscript and tables: AKH. All authors revised the manuscript for important intellectual content.

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REFERENCES

1 Aletaha D, Neogi T, Silman AJ, *et al*. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis* 2010;69:1580–8.
2 Padyukov L, Silva C, Stolt P, *et al*. A gene-environment interaction between smoking and shared epitope genes in HLA-DR provides a high risk of seropositive rheumatoid arthritis. *Arthritis Rheum* 2004;50:3085–92.
3 Costenbader KH, Feskanich D, Mandl LA, *et al*. Smoking intensity, duration, and cessation, and the risk of rheumatoid arthritis in women. *Am J Med* 2006;119:503.e1–503.e9.
4 Sugiyama D, Nishimura K, Tamaki K, *et al*. Impact of smoking as a risk factor for developing rheumatoid arthritis: a meta-analysis of observational studies. *Ann Rheum Dis* 2010;69:70–81.
5 Di Giuseppe D, Discacciati A, Orsini N, *et al*. Cigarette smoking and risk of rheumatoid arthritis: a dose-response meta-analysis. *Arthritis Res Ther* 2014;16:R61.
6 Makrygiannakis D, Hermansson M, Ulfgren AK, *et al*. Smoking increases peptidylarginine deiminase 2 enzyme expression in human lungs and increases citrullination in BAL cells. *Ann Rheum Dis* 2008;67:1488–92.
7 Klareskog L, Stolt P, Lundberg K, *et al*. A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination. *Arthritis Rheum* 2006;54:38–46.
8 Jaakkola JJ, Gissler M. Maternal smoking in pregnancy as a determinant of rheumatoid arthritis and other inflammatory polyarthropathies during the first 7 years of life. *Int J Epidemiol* 2005;34:664–71.
9 Söderlin MK, Andersson M, Bergman S. BARFOT study group. Second-hand exposure to tobacco smoke and its effect on disease activity in Swedish rheumatoid arthritis patients. Data from BARFOT, a multicenter study of RA. *Clin Exp Rheumatol* 2013;31:122–4.
10 Hedström AK, Stawiarz L, Klareskog L, *et al*. Smoking and susceptibility to rheumatoid arthritis in a Swedish population-based case-control study. *Eur J Epidemiol* 2018. Epub ahead of print.
11 Stolt P, Bengtsson C, Nordmark B, *et al*. Quantification of the influence of cigarette smoking on rheumatoid arthritis: results from a population based case-control study, using incident cases. *Ann Rheum Dis* 2003;62:835–41.
12 Internet-based information. http://www.scb.se (accessed 5 Nov 2017).

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EXTENDED REPORT

Biomechanical properties of bone are impaired in patients with ACPA-positive rheumatoid arthritis and associated with the occurrence of fractures

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ABSTRACT

Objectives Bone loss is a well-established consequence of rheumatoid arthritis (RA). To date, bone disease in RA is exclusively characterised by bone density measurements, while the functional properties of bone in RA are undefined. This study aimed to define the impact of RA on the functional properties of bone, such as failure load and stiffness.

Methods Micro-finite element analysis (µFEA) was carried out to measure failure load and stiffness of bone based on high-resolution peripheral quantitative CT data from the distal radius of anti-citrullinated protein antibody (ACPA)-positive RA (RA+), ACPA-negative RA (RA−) and healthy controls (HC). In addition, total, trabecular and cortical bone densities as well as microstructural parameters of bone were recorded. Correlations and multivariate models were used to determine the role of demographic, disease-specific and structural data of bone strength as well as its relation to prevalent fractures.

Results 276 individuals were analysed. Failure load and stiffness (both P<0.001) of bone were decreased in RA+, but not RA−, compared with HC. Lower bone strength affected both female and male patients with RA+, was related to longer disease duration and significantly (stiffness P=0.020; failure load P=0.012) associated with the occurrence of osteoporotic fractures. Impaired bone strength was correlated with altered bone density and microstructural parameters, which were all decreased in RA+. Multivariate models showed that ACPA status (P=0.007) and sex (P<0.001) were independently associated with reduced biomechanical properties of bone in RA.

Conclusion In summary, µFEA showed that bone strength is significantly decreased in RA+ and associated with fractures.

INTRODUCTION

Rheumatoid arthritis (RA) is an inflammatory joint disease associated with bone destruction and increased fracture risk.[1 2] While traditionally most attention in RA-related bone disease is drawn to periarticular bone erosions forming, the development of generalised bone loss in RA is of no less importance as it precipitates the increased fracture risk immanent to patients with RA.[3 4] Accumulating evidence suggests that bone loss in RA is driven to a large extent by the presence of anti-citrullinated protein antibodies (ACPA), which enhance osteoclastogenesis and thereby accelerate skeletal disease.[5–11] In support of this concept, there is good clinical evidence that local bone disease is more pronounced in patients with RA with ACPA[9 11] and also some evidence for more severe systemic bone loss.[10]

Although several studies have documented the loss of bone mass in patients with RA and some studies provided evidence for both cortical and trabecular bone loss in RA,[11] the functional impact of these structural changes for the stability of bone in RA are yet unknown. Hence, while we perceive that fracture risk is increased in RA, we do not really know whether the morphological changes of bone recorded in radiographic studies are indeed impacting the stability of bone. Micro-finite element analysis (µFEA) is a novel technique, which is increasingly used to characterise the biomechanical properties of bone and relate them to its microstructure.[12–14] This technique has been developed based on the availability of high-quality bone structure analyses in humans in vivo using high-resolution peripheral quantitative CT (HR-pQCT) scanners.[15–18] µFEA uses these data and mathematically models stiffness and failure load of the radius during a fall on the outstretched hand. Studies performed in healthy individuals have shown that µFEA accurately predicts bone strength and also allows better identification of individuals with fragility fractures than it can be achieved by the measurement of bone density.[14]

Hence, µFEA constitutes an attractive technology to better characterise the impact of bone changes in patients with systemic inflammatory diseases. ACPA-positive RA represents a paradigm disease to study µFEA as it is associated by changes in the bone microstructure and complicated by increased fracture risk. To characterise the mechanical properties of bone in RA, we applied µFEA in a cohort of patients with ACPA-positive RA (RA+) and ACPA-negative RA (RA−) and compared bone strength and stiffness of the distal radius of patients with RA+ and RA− with healthy controls. Furthermore, we aimed to define the demographic, disease-related and bone structural factors that are associated with bone strength in ACPA-positive RA. Finally, µFEA results were also related to fragility fractures in patients with ACPA-positive RA.

METHODS

Patients with RA and controls

Healthy controls (HC) and patients with RA were part of the Erlangen Imaging Cohort (ERIC), which

prospectively assesses bone composition in healthy individuals and patients with inflammatory arthritis.[19] In ERIC, 225 patients with RA were imaged in 2015 and 2016, 180 of them (80%) had motion grades 1–3 allowing proper analysis of bone structural parameters (16). The 180 analysed patients were representative for the entire cohort with similar sex, age and disease-specific parameters. All participants were recruited at the Department of Internal Medicine 3 of the University of Erlangen-Nuremberg and were clinically examined by an experienced rheumatologist (AK, JR, AJH). Patients with RA+ and RA− fulfilling the 2010 American College of Rheumatology/European League Against Rheumatism classification criteria were recruited. HC had to have (1) no signs of joint pain or swelling, (2) no presence of inflammatory or other chronic diseases, (3) no documented osteopaenia, osteoporosis or low-impact fracture or present/past use of bisphosphonates or prednisolone and (4) no positive test for autoantibodies such as ACPA or rheumatoid factor.[19] Demographic (age, sex, body mass index (BMI), smoking status) and disease-specific (disease duration, disease activity by Disease Activity Score-28, physical function by Health Assessment Questionnaire Disability Index, use of disease-modifying anti-rheumatic drugs (DMARDs), prednisolone and bisphosphonates, ACPA positivity and rheumatoid factor positivity) data were recorded. Fracture status was documented differentiating high-impact (sport injuries and accidents) from low-impact fractures (spontaneous or fall from walking or standing position). The study was conducted on approval of the local ethics committee of the University Clinic of Erlangen and with the authorisation of the National Radiation Safety Agency (Bundesamt für Strahlenschutz). Each individual provided informed consent.

HR-pQCT measurement

HR-pQCT was performed at the distal radius of the dominant hand by XtremeCT I scanner (Scanco Medical) using the manufacturer's default protocol for in vivo patient imaging. Measurements were carried out with an offset of 9.5 mm proximal to the reference line, which was manually set.[15 16 19] An anterior–posterior scout view determined the region of interest. One hundred eleven slices (82 µm nominal isotropic voxel size, 60 kVp effective energy, 900 µA) were taken. Standard analysis software (V.6.0) was used to determine the following density parameters: volumetric bone mineral density (vBMD) of total (Dtotal), trabecular (Dtrab), meta-trabecular (Dmeta), inner-trabecular (Dinn) and cortical bone (Dcomp (all in mg HA/cm^3)), ratio of meta-to-inner density (Meta/Inn, %) and cross-sectional bone area (mm^2).[15 16] Bone microstructure was evaluated by determining trabecular bone volume fraction (BV/TV, %), trabecular number (Tb.N (1/mm)), thickness (Tb.Th (mm)), separation (Tb.Sp (mm)), network inhomogeneity (SD of 1/trabecular number, Tb.1/N.SD (mm)) as well as cortical thickness (Ct.Th (mm)).[15 16]

Micro-finite element analysis

For µFEA, finite element analysis software (FAIM, V.8.0; Numerics88 Solution, Calgary, Canada) was used. In order to generate micro-finite element models, the segmented trabecular network and cortex of the HR-pQCT images were used.[20] Mesh size of the resulting models ranged from 1.5 to 3.5 million equally sized brick elements. Single linear isotropic tissue modelling was applied by assigning a tissue modulus of 6829 MPa and a Poisson's ratio of 0.3 homogeneously to each element.[13] A linear uniaxial compression test was simulated. Nodes on the proximal bone surface were fixed in z direction but unconstrained in x and y directions. Nodes on the distal bone surface were also free in x and y direction but exposed to a displacement equivalent to 1% strain along the z-axis.[13] Axial bone stiffness (kN/mm) as reaction force (RFz) divided by average displacement of the distal surface (Uz) and bone strength as estimated failure load (N) based on the Pistoia criterion was calculated.[12]

Statistical analysis

Statistical analyses were performed to test whether (1) groups (RA+, RA−, HC) were comparable with respect to demographic and disease-specific parameters, (2) bone strength and structural parameters differed among the groups and (3) sex and disease duration influence bone strength. In addition, we aimed to define independent factors associated with bone strength in the total population as well as in patients with RA+. Data were analysed using IBM SPSS V.21.0 (IBM, Armonk, New York, USA). Categorical variables are provided as numbers and percentages, continuous variables as mean±SD. Differences in frequency distributions of categorical variables were tested using χ^2 inferential tests. Assumptions of normally distributed continuous variables were tested using quantile–quantile plots as well as Kolmogorov-Smirnov and Shapiro-Wilk test. Clinical, bone structural and µFEA parameters were compared by using Kruskal-Wallis test (KW) with subsequent pairwise Mann-Whitney U tests, if KW test was significant. For correlating vBMD data with bone strength, Spearman's rank correlation coefficient (r_s) was used. In order to account for multiple testing, we applied Bonferroni-Holm adjustment for subgroup comparisons. Critical P values for adjusted levels of significance are shown in the corresponding tables. For all other comparisons, P values ≤0.05 were considered significant; all inferential tests were two-tailed. The direction of differences and relations is indicated by descriptive additional information or test coefficients.

To determine factors for bone strength in patients with RA, two multiple linear regression models using forced entry method for predictor inclusion were calculated in the RA patient subgroup. In the first model, we chose failure load as dependent variable, whereas in the second model stiffness was selected to be the dependent variable. In both models, sex, age, BMI, disease duration, biological DMARD use and ACPA status were entered as independent variables. In the third and fourth linear regression models, the total sample, consisting of healthy controls and patients with RA+ and RA−, was included, whereas failure load and stiffness, respectively, were selected as dependent variables while sex, age, BMI, biological DMARD use (for healthy controls set to no) and ACPA entity (dummy coded with healthy participants being the reference) were entered as independent variables. In regression models 3 and 4, information on ACPA status is reflected by the dummy coding procedure, that is, the coding scheme for RA+ versus HC is congruent to ACPA status in models 1 and 2.

RESULTS

Characteristics of patients and controls

A total of 276 Caucasian individuals (96 RA+, 84 RA− and 96 HC) were analysed. Age and sex distributions were not significantly different between the three groups. Smoking was more frequent among patients with RA+ (RA+: 24.0% vs HC: 7.3%, P=0.001), while BMI was higher in the RA+ (26.2±5.1; P=0.009) and RA− groups (26.8±5.8; P=0.007) than in HC (24.5±3.7). RA+ and RA− groups did not significantly differ in disease duration, disease activity, physical function and

Table 1 Demographic and clinical data of healthy controls and patients with rheumatoid arthritis

	HC	RA+	RA−
Demographic characteristics			
Sex (n male/n female)	38/58	26/70	22/62
Age (years; mean±SD)	50.1±16.5	54.2±12.2	53.9±12.2
BMI (mean±SD)	24.5±3.7*†	26.2±5.1*	26.8±5.8†
Smokers (n; %)	7 (7.3)*	23 (24.0)*	12 (14.3)
Disease-specific characteristics			
Disease duration (years; mean±SD)	–	9.7±8.9	7.1±7.2
DAS28-ESR (units; mean±SD)	–	3.3±1.6	3.2±1.3
HAQ-DI (units; mean±SD)	–	0.7±0.7	0.7±0.6
ACPA positive (n; %)	–	96 (100.0)‡	0 (0)‡
RF positive (n; %)	–	73 (76.0)‡	7 (8.3)‡
Anti-rheumatic treatment			
Glucocorticoids (n; %)	–	34 (35.4)	31 (36.9)
Methotrexate (n; %)	–	39 (40.6)	44 (52.4)
Other cDMARDs (n; %)	–	10 (10.4)	6 (7.1)
bDMARDs (n; %)	–	44 (45.8)	31 (36.9)
No current DMARD (n; %)§	–	22 (22.9)	21 (25.0)
Anti-osteoporotic treatment			
Vitamin D (n; %)	4 (4.2)*	36 (37.5)*‡	19 (22.6)‡
Bisphosphonates (n; %)	0*	8 (8.3)*	3 (3.6)

*Significance between HC vs RA+.
†Significance between HC vs RA−.
‡Significance between RA+ vs RA−.
§Either treatment naïve or in drug-free remission.
ACPA, anti-citrullinated protein antibody; bDMARD, biological disease-modifying anti-rheumatic drug; BMI, body mass index; cDMARD, conventional disease-modifying anti-rheumatic drug; DAS28-ESR, Disease Activity Score 28 - Erythrocyte Sedimentation Rate; HAQ-DI, Health Assessment Questionnaire Disability Index; HC, healthy controls; RA+, anti-citrullinated protein autoantibody-positive rheumatoid arthritis (RA); RA−, anti-citrullinated protein autoantibody-negative RA; RF, rheumatoid factor.

Table 2 Bone strength and structure in healthy controls and patients with rheumatoid arthritis

	HC	RA+	RA−
µFEA			
Stiffness, kN/mm	45.3±14.6*	36.4±13.9*†	41.5±12.5†
Failure load, N	2184±667*	1771±619*†	1986±579†
Bone structure (HR-pQCT)			
Volumetric bone mineral density			
Dtotal, mg HA/cm³	289±56*	256±58*†	286±61†
Dtrab, mg HA/cm³	164±37*‡	130±47*†	150±42†‡
Dmeta, mg HA/cm³	223±37*‡	196±43*†	207±41†‡
Dinn, mg HA/cm³	124±39*	98±44*	110±44
Dcomp, mg HA/cm³	786±66*‡	750±104*†	814±69†‡
Meta/Inn, %	1.96±0.65*	2.28±1.02*	2.06±0.66
Bone microstructure			
BV/TV, %	0.14±0.03*‡	0.12±0.04*†	0.12±0.03†‡
Tb.N, 1/mm	2.04±0.30*‡	1.81±0.42*†	1.93±0.37†‡
TbTh, mm	0.07±0.01*	0.06±0.01*	0.06±0.01
Tb.Sp, mm	0.44±0.11*‡	0.54±0.23*†	0.49±0.21†‡
Tb.1/N.SD, mm	0.19±0.07*‡	0.29±0.24*	0.23±0.15[b]
Ct.Th, mm	0.67±0.18*	0.59±0.21*†	0.69±0.19†

Bonferroni-Holm adjustment: critical P values indicating significant results for all investigated parameters were as follows: P1=0.0167, P2=0.025, P3=0.05.
*Significance between HC vs RA+.
†Significance between RA+ vs RA−.
‡Significance between HC vs RA−.
BV/TV, trabecular bone volume per tissue volume; Ct.Th, cortical thickness; Dcomp, compact (cortical) volumetric bone mineral density (vBMD); Dinn, inner trabecular vBMD; Dmeta, meta-trabecular vBMD; Dtotal, total vBMD; Dtrab, trabecular vBMD; HC, healthy controls; HR-pQCT, high-resolution peripheral CT; Meta/Inn, ratio of meta-to-inner density; RA+, anti-citrullinated protein autoantibody-positive rheumatoid arthritis (RA); RA−, anti-citrullinated protein autoantibody-negative RA; Tb.1/N.SD, inhomogeneity of network; Tb.N, trabecular number; Tb.Sp, trabecular separation; Tb.Th, trabecular thickness; µFEA, micro-finite element analysis.

DMARD treatment. Detailed information on demographic and disease-specific characteristics are listed in table 1.

Bone strength is reduced in patients with RA+

We first analysed whether bone strength is impaired in patients with RA+ and patients with RA− compared with HC. When assessing bone biomechanical properties by µFEA, patients with RA+, but not RA−, showed significantly lower stiffness and failure load compared with HC (stiffness: 36.4±13.9 vs 45.3±14.6 kN/mm, P<0.001; failure load: 1771±619 vs 2184±667 N, P<0.001) (table 2, figure 1). Furthermore, when patients with RA+ were compared with patients with RA−, stiffness and failure load were significantly decreased in patients with RA+ (figure 1, table 2). Figure 2 shows representative images of µFEA analysis in RA+, RA− and HC.

Volumetric bone mineral density and bone microstructure are reduced in patients with RA+

We next compared structural bone parameters between the groups. Total, trabecular and cortical bone mineral densities (vBMD in mg HA/cm³) were decreased in patients with RA+ compared with HC (total vBMD: 256±58 vs 289±56, P<0.001; trabecular vBMD: 130±47 vs 164±37, P<0.001; cortical vBMD: 750±104 vs 786±656, P=0.021) with statistical significance. Microstructure analysis revealed significantly lower trabecular number (1.8±0.4 vs 2.0±0.3 1/mm, P<0.001) and thickness (0.06±0.01 vs 0.07±0.01 mm, P=0.025) in RA+. In addition, also cortical thickness was lower in patients with RA+ than in HC (0.59±0.21 vs 0.67±0.18 mm, P=0.012). Bone structure in RA− was fundamentally different: First, cortical vBMD was even higher in RA− than in HC. In addition, while trabecular vBMD was decreased in RA− compared with HC, trabecular bone loss was less pronounced than in RA+. Finally, total vBMD in RA− was not reduced as compared with HC (table 2).

Correlation between bone strength and structure in RA

We hypothesised that bone strength is related to structure in patients with RA. Indeed, failure load in patients with RA+ (r_s=0.65; r_s=0.64), like in patients with RA− (r_s=0.58; r_s=0.74) and HC (r_s=0.44, r_s=0.62), was significantly (all P<0.001) correlated to total vBMD and trabecular vBMD, respectively. Stiffness of bone was also significantly (all P<0.001) related to total (RA+: r_s=0.68, RA−: r_s=0.62, HC: r_s=0.47) and trabecular vBMD (RA+: r_s=0.61 RA−: r_s=0.75, HC: r_s=0.63). Interestingly, however, only patients with RA showed a correlation between bone strength and cortical vBMD (RA+: r_s=0.24, P=0.021; RA−: r_s=0.28, P=0.009), while there was no significant correlation in HC.

Sex effects on bone strength in RA

We then characterised sex-dependent differences of bone strength in RA. Failure load (2331±584 vs 1563±492 n, P<0.001) and stiffness (48.0±13.5 vs 32.1±11.4 kN/mm, P<0.001) were

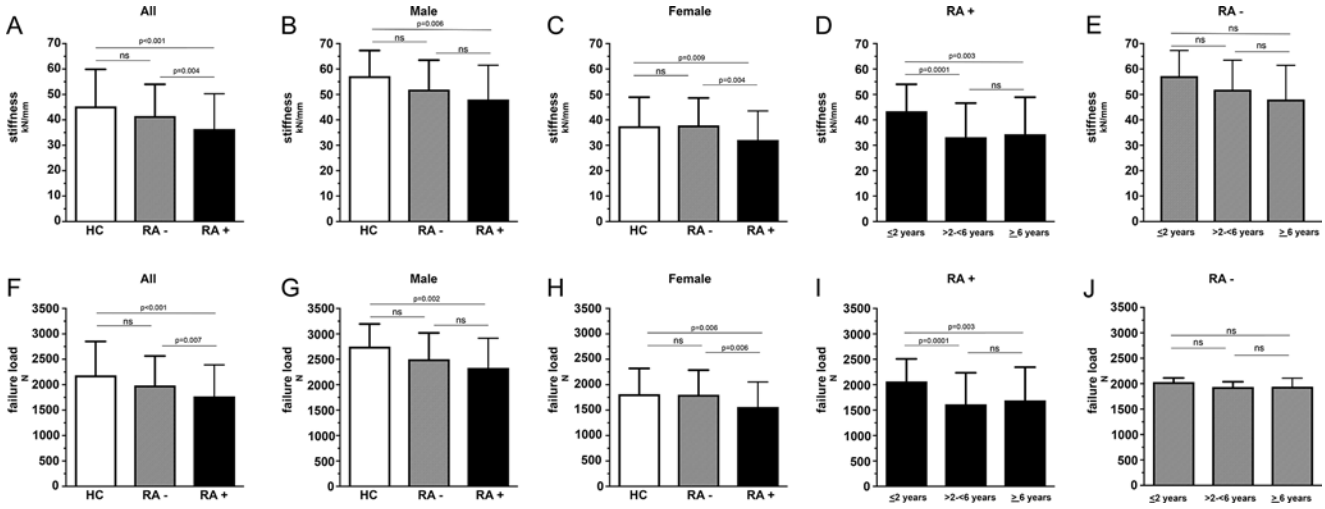

Figure 1 Comparison of bone strength parameters between healthy controls, patients with anti-citrullinated protein antibody (ACPA)-negative (RA–) and ACPA-positive (RA+) rheumatoid arthritis (RA) and impact of disease duration. Axial stiffness (upper row) and failure load (bottom row) are shown as mean±SD. Comparison between healthy controls (HC, white bars) and RA (RA–, grey/striped bars; RA+, black bars) are shown in A and F; between healthy men and men with RA in B and G; between healthy women and RA in C and H. Mean±SD values of three different RA+ disease duration subgroups are depicted in column D and H; for RA– in E and J.

significantly higher in men with RA+ than in women with RA+. Similar differences, although at an overall higher level, were also found in HC and RA− with men showing higher failure load and stiffness than women. Even more importantly, healthy men showed significantly stiffer (57.2±10.1 kN/mm; P=0.006) and stronger (2752±445 N; P=0.002) bones than men with RA+ (48.0±13.5 kN/mm and 2331±584 N), respectively (figure 1). Furthermore, also healthy women showed significantly stiffer (37.5±11.4 kN/mm; P=0.009) and stronger bones (1813±508 N; P=0.006) than women with RA+ (table 3). No such differences were found in RA−. Bone size (cross-sectional area) was not different between RA+ (340±56 mm^2) and HC (326±86 mm^2) but was related to sex in RA+ (men: 421±75 mm^2 vs women: 310±87 mm^2, P<0.001) and HC (men: 398±81 mm^2 vs women: 279±50 mm^2, P<0.001). Detailed information on density and microstructural parameters of HC and patients with RA and their relation to sex are listed in table 3.

Impact of disease duration on bone strength

We next analysed whether bone strength in RA is associated with disease duration. We compared stiffness and failure load in three groups of patients with RA+ and RA− with different disease durations (≤2 years: n=24; >2−<6 years: n=18; ≥6 years: n=54). In patients with RA+, stiffness of bone significantly declined with disease duration from 43.4±10.6 kN/mm (≤2 years) to 33.2±13.4 kN/mm (>2−<6 years; P=0.001) and 34.4±14.5 kN/mm (≥6 years: P=0.003). Similarly,

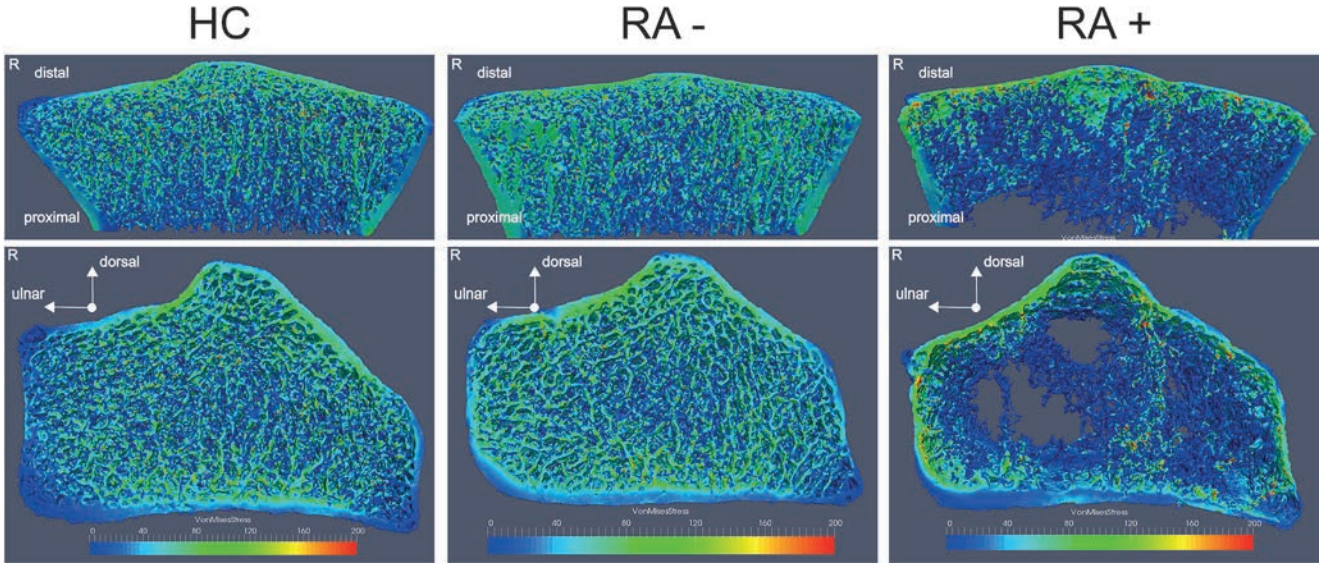

Figure 2 Depiction of a finite element analysis-derived stress distribution image of a healthy control (HC) and anti-citrullinated protein antibody (ACPA)-negative (RA–) and ACPA-positive (RA+) rheumatoid arthritis (RA). Right and middle column display the right radius of a female patient with RA+ and RA–. Left column shows a gender-comparable and age-comparable HC. For comparison, full µFEA models (bottom) and cut through the radial bone (top) are shown to reveal differences in stress distribution for cortical and trabecular network. Colour map labels the von Mises stress (MPa) for described loading scenario.

Table 3 Comparison of bone strength and structure in male and female healthy controls and patients with rheumatoid arthritis

	HC (n=96)		RA+ (n=96)		RA− (n=84)	
Groups	**Male (n=38)**	**Female (n=58)**	**Male (n=26)**	**Female (n=70)**	**Male (n=22)**	**Female (n=62)**
μFEA						
Stiffness, kN/mm	57.2±10.1*†	37.5±11.4†	48.0±13.5*	32.1±11.4‡	51.9±11.6*	37.8±10.8
Failure load, N	2752±445*†	1813±508†	2331±584*	1563±492‡	2502±517*	1803±484
Bone structure (HR-pQCT)						
Volumetric bone mineral density						
Dtotal, mg HA/cm³	301±52†	282±57	259±50	255±61‡	288±56	286±63
Dtrab, mg HA/cm³	185±28*†	151±36†	145±42	124±47‡	170±30*	142±43
Dmeta, mg HA/cm³	240±30*†	211±37†	212±3	191±46	227±25*	200±43
Dinn, mg HA/cm³	147±27*†	109±38†	116±4*	291±43	131±16*	102±44
Dcomp, mg HA/cm³	767±51*	799±71	734±91	756±108‡	776±77*	827±62
Meta/Inn, %	1.66±0.17*	2.15±0.7	2.17±1.3	2.32±0.93	1.86±0.47	2.14±0.70
Bone microstructure						
BV/TV, %	0.15±0.02*†	0.13±0.03	0.13±0.03*	0.11±0.04	0.14±0.03*	0.12±0.04
Tb.N, 1/mm	2.18±0.21*	1.95±0.32†	1.93±0.42*	1.76±0.41	2.17±0.27*	1.84±0.37
Tb.Th, mm	0.07±0.01*	0.06±0.01	0.07±0.01	0.06±0.01	0.07±0.01	0.06±0.01
Tb.Sp, mm	0.39±0.04*	0.47±0.13†	0.49±0.18	0.55±0.24	0.40±0.06*	0.52±0.23
Tb.1/N.SD, mm	0.16±0.03	0.20±0.08†	0.27±0.22	0.29±0.25	0.18±0.06	0.25±0.17
Ct.Th, mm	0.70±0.17	0.64±0.18	0.61±0.21	0.58±0.21‡	0.70±0.23	0.69±0.18

Bonferroni-Holm adjustment: critical P values indicating significant results for all investigated parameters were as follows: P1=0.0056, P2=0.0063, P3=0.0071, P4=0.0083, P5=0.01, P6=0.0125, P7=0.0167, P8=0.025, P9=0.05.
*Significance between men vs women in the same group.
†Significance between HC vs RA+ of same sex.
‡Significance between RA+ vs RA− of same sex.
§Significance between HC vs RA− of same sex.
BV/TV, trabecular bone volume per tissue volume; Ct.Th, cortical thickness; Dcomp, compact (cortical) volumetric bone mineral density (vBMD); Dinn, inner trabecular vBMD; Dmeta, meta-trabecular vBMD; Dtotal, total vBMD; Dtrab, trabecular vBMD; HC, healthy controls; HR-pQCT, high-resolution peripheral CT; Meta/Inn, ratio of meta-to-inner density; RA+, anti-citrullinated protein autoantibody-positive rheumatoid arthritis (RA); RA−, anti-citrullinated protein autoantibody-negative RA; Tb.1/N.SD, inhomogeneity of network; Tb.N, trabecular number; Tb.Sp, trabecular separation; Tb.Th, trabecular thickness; μFEA, micro-finite element analysis.

failure load of bone declined from 2065±443 N (≤2 years) to 1615±619 N (>2−<6 years; P=0.001) and 1693±652 N (≥6 years: P=0.003) (table 4, figure 1). No such decline of stiffness and failure load was found in RA− (table 4). Furthermore, age of patients with RA+ with ≤2 years in disease duration did not differ from the ones with >2 years in disease duration. In accordance with the decline of biomechanical properties, also the volumetric and microstructural characteristics of bone declined with disease duration. Hence, especially volumetric BMD and microstructural parameters of the trabecular bone continuously decreased the longer patients with RA+ had been afflicted by disease (table 4).

Lower bone strength in patients with RA+ with low-impact fractures

We hypothesised that bone strength in patients with RA is associated with prevalent fractures and particularly focused on low-impact fractures that occurred after the diagnosis of RA. Although the absolute number of patients with RA+ with fractures was limited (n=9), those patients had significantly lower bone stiffness (28.0±9.4 kN/mm; P=0.020) and failure load (1374±412 N; P=0.012) along with lower trabecular vBMD (99±33; P=0.012) compared with patients with RA+ without fractures (stiffness: 39.1±14.2 kN/mm; failure load: 1890±638 N; trabecular vBMD: 138±45) suggesting that poor biomechanical properties of bone are associated with fracture. In contrast, very few fractures (n=3) occurred in patients with RA− with no association to bone strength.

Factors determining bone strength in RA

In order to search for parameters that influence failure load in patients with RA, we calculated linear regression models with sex, age, BMI, disease duration, biological DMARD use and ACPA status as independent variables. The first model accounted for 31.9% of the variance of failure load. Sex (P<0.001), age (P=0.040) and ACPA status (P=0.007) were independently associated with failure load of bone in RA. We calculated a similar model for bone stiffness using the same independent variables. This model accounted for a similar amount of variance (28.3%), again showing sex (P<0.001), age (P=0.038) and ACPA status (P=0.007) to be negatively and independently associated with reduced biomechanical properties of bone in patients with RA.

Two further regression models of failure load and stiffness including the total sample accounted for 43.9% of the variance of failure load and 40.4% of the variance of stiffness. In both models, sex, age and RA+ versus HC (ie, ACPA positivity) were negatively associated with the dependent variables (all P≤0.006). All results of regression models are depicted in online supplementary table 1.

DISCUSSION

The vast majority of knowledge on systemic bone loss in RA comes from dual energy X-ray absorptiometry studies, which do not take into account potential changes of bone microstructure. More recent analyses supported the notion that RA is characterised by substantial impairment of bone microstructure suggesting that its biomechanical properties may indeed

Table 4 Impact of RA disease duration on bone strength and structure

	RA+			RA−		
Disease duration	**≤2 years**	**>2–<6 years**	**≥6 years**	**≤2 years**	**>2–<6 years**	**≥6 years**
N	24	18	54	43	21	20
Age, years	49.2±14.4	54.6±11.6	56.3±10.8	49.7±12.6*†	57.5±11.9	59.0±8.3
Sex, n male/n female	9/15	4/14	13/41	9/34	7/14	6/14
µFEA						
Stiffness, kN/mm	43.4±10.6*†	33.2±13.4	34.4±14.5	42.6±12.0	40.2±10.2	40.5±16.0
Failure load, N	2065±433*†	1615±620	1693±652	2023±542	1935±475	1942±754
Bone structure (HR-pQCT)						
Volumetric bone mineral density						
Dtotal, mg HA/cm³	283±55†	251±60	246±57	292±56	284±53	276±79
Dtrab, mg HA/cm³	165±40†	133±42	113±42	152±37	155±30	139±58
Dmeta, mg HA/cm³	223±35*†	191±41	186±43	210±34	213±33	195±58
Dinn, mg HA/cm³	129±36*†	95±41	85±42	112±41	114±30	100±59
Dcomp, mg HA/cm³	772±98	752±100	740±106	830±63	802±74	793±74
Meta/Inn, %	1.79±0.35*†	2.25±0.79	2.50±1.21	2.04±0.51	1.95±0.41	2.24±1.07
Bone microstructure						
BV/TV, %	0.14±0.03*†	0.11±0.03	0.11±0.03	0.13±0.03	0.13±0.03	0.12±0.05
Tb.N, 1/mm	2.04±0.30*†	1.70±0.48	1.74±0.41	1.96±0.24	2.01±0.30	1.75±0.58
Tb.Th, mm	0.07±0.01†	0.07±0.01‡	0.06±0.01	0.06±0.01	0.06±0.01	0.06±0.01
Tb.Sp, mm	0.43±0.09*†	0.57±0.20	0.57±0.27	0.45±0.07	0.44±0.08	0.61±0.40
Tb.1/N.SD, mm	0.19±0.06*†	0.34±0.27	0.31±0.28	0.20±0.05	0.19±0.06	0.34±0.28
Ct.Th, mm	0.61±0.20	0.56±0.22	0.59±0.21	0.71±0.18	0.66±0.18	0.66±0.24

Bonferroni-Holm adjustment: critical P values indicating significant results for all investigated parameters were as follows: P1=0.0167, P2=0.025, P3=0.05.
*Significance between ≤2 years' and >2–<6 years' disease duration.
†Significance between ≤2 years' and ≥6 years' disease duration.
‡Significance between >2–<6 years' and ≥6 years' disease duration.
BV/TV, trabecular bone volume per tissue volume; Ct.Th, cortical thickness; Dcomp, compact (cortical) volumetric bone mineral density (vBMD); Dinn, inner trabecular vBMD; Dmeta, meta-trabecular vBMD; Dtotal, total vBMD; Dtrab, trabecular vBMD; HR-pQCT, high-resolution peripheral CT; Meta/Inn, ratio of meta-to-inner density; RA+, anti-citrullinated protein autoantibody positive rheumatoid arthritis (RA); RA−, anti-citrullinated protein autoantibody negative RA; Tb.N, trabecular number; Tb.1/N.SD, inhomogeneity of network; Tb.Sp, trabecular separation; Tb.Th, trabecular thickness; µFEA, micro-finite element analysis.

be significantly altered. Nonetheless, the biomechanical properties of bone in patients with RA have not been characterised. This study now clearly shows that failure load and stiffness of bone are significantly impaired in both female and male patients with RA.

We used µFEA to define the biomechanical properties of bone in RA, which is currently the most advanced method to define the functional qualities of bone. To date, µFEA has been largely applied in healthy individuals,[21–29] where fracture risk association has been described,[27–29] and small cohorts of various non-inflammatory diseases such as Turner syndrome,[30] type 1 diabetes,[31] male osteoporosis,[32] idiopathic scoliosis,[33] chronic obstructive lung disease[34] and end-stage renal failure.[35] In contrast, biomechanical properties of bone in inflammatory diseases, especially in RA, remained inadequately characterised. While one small study found reduced bone strength in patients with ankylosing spondylitis,[36] another study performed in RA failed to show such changes.[37] However, these latter data need to be seen with caution as they are based on a rather small number of patients, who were characterised by surprisingly high bone mass—potentially based on their ethnic background, which makes differentiation from controls challenging.[38] Furthermore, the study lacked important disease characteristics of RA such as ACPA status, which is essential since ACPA have shown to play a causal role in RA-related bone loss by inducing osteoclast differentiation.[5–7]

In our study comprising more than 250 patients and controls, bone strength was significantly reduced in patients with RA+. Our analyses showed that only patients with RA+ but not patients with RA− had a lower failure load and stiffness underlining the concept that patients with RA+ are a distinct population with respect to genetic background, pathogenesis and clinical manifestation of the disease.[1] The differences in bone strength between patients with RA+ and RA− are likely based on the previously shown functional properties of ACPA and RF in inducing osteoclast differentiation[5 6 39] and provide solid clinical evidence that the bone composition and strength in patients with RA depends on the presence of autoantibodies. In accordance and reflecting our previous data total, trabecular and cortical volumetric bone mineral densities as well as microstructural parameters of bone were all reduced in patients with RA+.[11] Importantly, the differences in bone strength between RA+ and HC groups were found in both women and men and were related to disease duration. Most strikingly, however, low failure load and stiffness in patients with RA+ were associated with higher prevalence of osteoporotic fractures. This latter finding suggests that measurement of bone strength identified patients with RA at risk for fragility fracture.

Total, trabecular and cortical volumetric bone density in patients with RA+ were lower than those previously described in ankylosing spondylitis,[36 40] inflammatory bowel diseases,[41] psoriatic arthritis and psoriasis[42] or even osteogenesis imperfecta.[43] Similarl compromised bone was only described in postmenopausal women with fractures[16 28 44] and for men with pathological fractures.[32] Notably, however, patients with RA+ in our study were approximately 20 years younger than the participants included in the aforementioned studies. Published normative data on radial bones from healthy individuals measured by HR-pQCT also show substantially higher bone densities for comparable ages.[15 45] From these normative data, it appears that bone in patients with RA+

has adopted the properties that are characteristic for the bone of a healthy individual 20 years older.

In summary, this study shows that bone strength is significantly reduced in both female and male patients with RA+ and associated with the development of osteoporotic fractures. Reduced bone strength in patients with RA+ results from profound changes in bone volumetric density and microarchitecture resembling the structural features of bone of a healthy individual 20 years older.

Contributors FS, DS, AK, AJH and JR collected the data. AK, GS, DS, FS, KE and A-ML analysed and interpreted the data. FS, DS, AK and GS prepared and revised the manuscript. AK, DS and GS designed the study.

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Competing interests None declared.

Patient consent Obtained.

Ethics approval The study was conducted upon approval of the local ethics committee of the University Clinic of Erlangen and with the authorisation of the National Radiation Safety Agency (Bundesamt für Strahlenschutz).

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REFERENCES

1 McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. *N Engl J Med* 2011;365:2205–19.
2 Schett G, Gravallese E. Bone erosion in rheumatoid arthritis: mechanisms, diagnosis and treatment. *Nat Rev Rheumatol* 2012;8:656–64.
3 van Staa TP, Geusens P, Bijlsma JW, *et al*. Clinical assessment of the long-term risk of fracture in patients with rheumatoid arthritis. *Arthritis Rheum* 2006;54:3104–12.
4 Chen B, Cheng G, Wang H, *et al*. Increased risk of vertebral fracture in patients with rheumatoid arthritis: A meta-analysis. *Medicine* 2016;95:e5262.
5 Harre U, Georgess D, Bang H, *et al*. Induction of osteoclastogenesis and bone loss by human autoantibodies against citrullinated vimentin. *J Clin Invest* 2012;122:1791–802.
6 Krishnamurthy A, Joshua V, Haj Hensvold A, *et al*. Identification of a novel chemokine-dependent molecular mechanism underlying rheumatoid arthritis-associated autoantibody-mediated bone loss. *Ann Rheum Dis* 2016;75:721–9.
7 Engdahl C, Bang H, Dietel K, *et al*. Periarticular bone loss in arthritis is induced by autoantibodies against citrullinated vimentin. *J Bone Miner Res* 2017;32:1681–91.
8 Mathsson L, Mullazehi M, Wick MC, *et al*. Antibodies against citrullinated vimentin in rheumatoid arthritis: higher sensitivity and extended prognostic value concerning future radiographic progression as compared with antibodies against cyclic citrullinated peptides. *Arthritis Rheum* 2008;58:36–45.
9 van Gaalen FA, Linn-Rasker SP, van Venrooij WJ, *et al*. Autoantibodies to cyclic citrullinated peptides predict progression to rheumatoid arthritis in patients with undifferentiated arthritis: a prospective cohort study. *Arthritis Rheum* 2004;50:709–15.
10 Bugatti S, Bogliolo L, Vitolo B, *et al*. Anti-citrullinated protein antibodies and high levels of rheumatoid factor are associated with systemic bone loss in patients with early untreated rheumatoid arthritis. *Arthritis Res Ther* 2016;18:226.
11 Kocijan R, Finzel S, Englbrecht M, *et al*. Differences in bone structure between rheumatoid arthritis and psoriatic arthritis patients relative to autoantibody positivity. *Ann Rheum Dis* 2014;73:2022–8.
12 Pistoia W, van Rietbergen B, Lochmüller EM, *et al*. Estimation of distal radius failure load with micro-finite element analysis models based on three-dimensional peripheral quantitative computed tomography images. *Bone* 2002;30:842–8.
13 Macneil JA, Boyd SK. Bone strength at the distal radius can be estimated from high-resolution peripheral quantitative computed tomography and the finite element method. *Bone* 2008;42:1203–13.
14 Engelke K, van Rietbergen B, Zysset P. FEA to measure bone strength: a review. *Clin Rev Bone Miner Metab* 2016;14:26–37.
15 Khosla S, Riggs BL, Atkinson EJ, *et al*. Effects of sex and age on bone microstructure at the ultradistal radius: a population-based noninvasive in vivo assessment. *J Bone Miner Res* 2006;21:124–31.
16 Boutroy S, Bouxsein ML, Munoz F, *et al*. In vivo assessment of trabecular bone microarchitecture by high-resolution peripheral quantitative computed tomography. *J Clin Endocrinol Metab* 2005;90:6508–15.
17 Buie HR, Campbell GM, Klinck RJ, *et al*. Automatic segmentation of cortical and trabecular compartments based on a dual threshold technique for in vivo micro-CT bone analysis. *Bone* 2007;41:505–15.
18 MacNeil JA, Boyd SK. Load distribution and the predictive power of morphological indices in the distal radius and tibia by high resolution peripheral quantitative computed tomography. *Bone* 2007;41:129–37.
19 Simon D, Kleyer A, Stemmler F, *et al*. Age- and sex-dependent changes of intra-articular cortical and trabecular bone structure and the effects of rheumatoid arthritis. *J Bone Miner Res* 2017;32.
20 van Rietbergen B, Weinans H, Huiskes R, *et al*. A new method to determine trabecular bone elastic properties and loading using micromechanical finite-element models. *J Biomech* 1995;28:69–81.
21 Shanbhogue VV, Brixen K, Hansen S. Age- and sex-related changes in bone microarchitecture and estimated strength: a three-year prospective study using HRpQCT. *J Bone Miner Res* 2016;31:1541–9.
22 Vilayphiou N, Boutroy S, Sornay-Rendu E, *et al*. Age-related changes in bone strength from HR-pQCT derived microarchitectural parameters with an emphasis on the role of cortical porosity. *Bone* 2016;83:233–40.
23 Macdonald HM, Nishiyama KK, Kang J, *et al*. Age-related patterns of trabecular and cortical bone loss differ between sexes and skeletal sites: a population-based HR-pQCT study. *J Bone Miner Res* 2011;26:50–62.
24 Dalzell N, Kaptoge S, Morris N, *et al*. Bone micro-architecture and determinants of strength in the radius and tibia: age-related changes in a population-based study of normal adults measured with high-resolution pQCT. *Osteoporos Int* 2009;20:1683–94.
25 Hansen S, Shanbhogue V, Folkestad L, *et al*. Bone microarchitecture and estimated strength in 499 adult Danish women and men: a cross-sectional, population-based high-resolution peripheral quantitative computed tomographic study on peak bone structure. *Calcif Tissue Int* 2014;94:269–81.
26 Melton LJ, Riggs BL, van Lenthe GH, *et al*. Contribution of in vivo structural measurements and load/strength ratios to the determination of forearm fracture risk in postmenopausal women. *J Bone Miner Res* 2007;22:1442–8.
27 Vilayphiou N, Boutroy S, Sornay-Rendu E, *et al*. Finite element analysis performed on radius and tibia HR-pQCT images and fragility fractures at all sites in postmenopausal women. *Bone* 2010;46:1030–7.
28 Boutroy S, Van Rietbergen B, Sornay-Rendu E, *et al*. Finite element analysis based on in vivo HR-pQCT images of the distal radius is associated with wrist fracture in postmenopausal women. *J Bone Miner Res* 2008;23:392–9.
29 Stein EM, Silva BC, Boutroy S, *et al*. Primary hyperparathyroidism is associated with abnormal cortical and trabecular microstructure and reduced bone stiffness in postmenopausal women. *J Bone Miner Res* 2013;28:1029–40.
30 Hansen S, Brixen K, Gravholt CH. Compromised trabecular microarchitecture and lower finite element estimates of radius and tibia bone strength in adults with Turner syndrome: a cross-sectional study using high-resolution-pQCT. *J Bone Miner Res* 2012;27:1794–803.
31 Shanbhogue VV, Hansen S, Frost M, *et al*. Bone geometry, volumetric density, microarchitecture, and estimated bone strength assessed by HR-pQCT in adult patients with type 1 diabetes mellitus. *J Bone Miner Res* 2015;30:2188–99.
32 Vilayphiou N, Boutroy S, Szulc P, *et al*. Finite element analysis performed on radius and tibia HR-pQCT images and fragility fractures at all sites in men. *J Bone Miner Res* 2011;26:965–73.
33 Cheuk KY, Zhu TY, Yu FW, *et al*. Abnormal bone mechanical and structural properties in adolescent idiopathic scoliosis: a study with finite element analysis and structural model index. *Calcif Tissue Int* 2015;97:343–52.
34 Romme EA, Rutten EP, Geusens P, *et al*. Bone stiffness and failure load are related with clinical parameters in men with chronic obstructive pulmonary disease. *J Bone Miner Res* 2013;28:2186–93.
35 Trombetti A, Stoermann C, Chevalley T, *et al*. Alterations of bone microstructure and strength in end-stage renal failure. *Osteoporos Int* 2013;24:1721–32.
36 Nigil Haroon N, Szabo E, Raboud JM, *et al*. Alterations of bone mineral density, bone microarchitecture and strength in patients with ankylosing spondylitis: a cross-sectional study using high-resolution peripheral quantitative computerized tomography and finite element analysis. *Arthritis Res Ther* 2015;17:377.
37 Zhu TY, Griffith JF, Qin L, *et al*. Structure and strength of the distal radius in female patients with rheumatoid arthritis: a case–control study. *J Bone Miner Res* 2013;28:794–806.
38 Popp KL, Hughes JM, Martinez-Betancourt A, *et al*. Bone mass, microarchitecture and strength are influenced by race/ethnicity in young adult men and women. *Bone* 2017;103:200–8.
39 Harre U, Lang SC, Pfeifle R, *et al*. Glycosylation of immunoglobulin G determines osteoclast differentiation and bone loss. *Nat Commun* 2015;6:6651.

40 Klingberg E, Lorentzon M, Göthlin J, *et al*. Bone microarchitecture in ankylosing spondylitis and the association with bone mineral density, fractures, and syndesmophytes. *Arthritis Res Ther* 2013;15:R179.
41 Haschka J, Hirschmann S, Kleyer A, *et al*. High-resolution quantitative computed tomography demonstrates structural defects in cortical and trabecular bone in IBD patients. *J Crohns Colitis* 2016;10:532–40.
42 Kocijan R, Englbrecht M, Haschka J, *et al*. Quantitative and qualitative changes of bone in psoriasis and psoriatic arthritis patients. *J Bone Miner Res* 2015;30:1775–83.
43 Kocijan R, Muschitz C, Haschka J, *et al*. Bone structure assessed by HR-pQCT, TBS and DXL in adult patients with different types of osteogenesis imperfecta. *Osteoporos Int* 2015;26:2431–40.
44 Sornay-Rendu E, Boutroy S, Munoz F, *et al*. Alterations of cortical and trabecular architecture are associated with fractures in postmenopausal women, partially independent of decreased BMD measured by DXA: the OFELY study. *J Bone Miner Res* 2007;22:425–33.
45 Burt LA, Liang Z, Sajobi TT, *et al*. Sex- and site-specific normative data curves for HR-pQCT. *J Bone Miner Res* 2016;31:2041–7.

EXTENDED REPORT

High erythrocyte levels of the n-6 polyunsaturated fatty acid linoleic acid are associated with lower risk of subsequent rheumatoid arthritis in a southern European nested case–control study

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ABSTRACT

Objectives Findings relating to dietary intake of n-3 polyunsaturated fatty acids (PUFA) and risk of rheumatoid arthritis (RA) are mixed. Erythrocyte membrane PUFA is an accurate objective biomarker of PUFA status; however, there are little data on erythrocyte membrane PUFA and risk of RA. The objective was therefore to compare erythrocyte membrane PUFA between pre-RA individuals and matched controls from a population-based sample, and specifically to test the hypothesis that higher levels of longer chain n-3 PUFA are associated with lower risk of RA.

Methods The European Prospective Investigation into Cancer and Nutrition (EPIC) is a large European prospective cohort study of apparently healthy populations. We undertook a nested case–control study by identifying RA cases with onset after enrolment (pre-RA) in four EPIC cohorts in Italy and Spain. Confirmed pre-RA cases were matched with controls by age, sex, centre, and date, time and fasting status at blood collection. Conditional logistic regression analysis was used to estimate associations of PUFA with the development of RA, adjusting for potential confounders including body mass index, waist circumference, education level, physical activity, smoking status and alcohol intake.

Results The study analysed samples from 96 pre-RA subjects and 258 matched controls. In this analysis, the median time to diagnosis (defined as time between date of blood sample and date of diagnosis) was 6.71 years (range 0.8–15). A significant inverse association was observed with n-6 PUFA linoleic acid (LA) levels and pre-RA in the fully adjusted model (highest tertile: OR 0.29; 95% CI 0.12 to 0.75; P for trend 0.01). No association was observed with any individual n-3 PUFA, total n-3 PUFA or total n-3:n-6 ratio.

Conclusions Erythrocyte levels of the n-6 PUFA LA were inversely associated with risk of RA, whereas no associations were observed for other n-6 or n-3 PUFA. Further work is warranted to replicate these findings and to investigate if lower LA levels are a bystander or contributor to the process of RA development.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease characterised by inflammation of synovial joints, with a prevalence worldwide of 0.5%–1%. Environmental/non-genetic susceptibility factors account for up to 60% of the risk for developing RA[1]; however, only a limited number of such factors have been identified.[2 3] Long-chain n-3 (also known as omega-3) polyunsaturated fatty acids (PUFA), especially eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3), have long been considered to have anti-inflammatory and immunomodulatory actions.[4] Neither n-3 nor n-6 (omega 6) series PUFA (each family is determined by the number of carbon atoms between the methyl end of the fatty acyl chain and the first double-bond) can be synthesised de novo by large animals and so they are considered essential. The main dietary form of n-3 PUFA in most humans is the plant-derived alpha-linolenic acid (ALA; 18:3 n-3).[5] ALA is metabolised by a series of desaturation and elongation steps to the longer chain EPA and DHA. This process of conversion into longer chain PUFA is poor in humans[6] and in direct competition with the desaturation and elongation of the considerably more abundant n-6 PUFA. Longer chain n-3 PUFAs can be synthesised by phytoplankton and are passed up the food chain through zooplankton to fish. Because of limited synthesis from ALA in humans, most EPA and DHA in the human body are of dietary origin, with marine foods such as deep sea fatty fish or fish oil supplements being particularly good sources. The parent n-6 PUFA is linoleic acid (LA; 18:2 n-6), which is more commonly obtained in the diet than any n-3 PUFA, with vegetable oils, especially sunflower, corn and soybean, being particularly good sources. Desaturation and elongation of LA give rise to longer chain n-6 PUFA such as arachidonic acid and gamma-linolenic acid. Although there is a widespread conception that n-6 PUFAs are proinflammatory, some data suggest an immunomodulatory potential.[7]

Fish oil ameliorates collagen-induced arthritis,[8 9] and has shown efficacy in RA clinical trials.[10–12] While the observed effects of fish oil in established RA are

modest, the concept of a 'window of opportunity' in early disease suggests a greater potential for protective immunomodulation in the very earliest stages. Findings on the relationship between dietary intake of fish/fish oil and prevention of RA have been inconsistent.[13–17] It is usually assumed that any observable effects of fish intake are due to their long-chain n-3 PUFA content. One study reported that higher intake of n-3 PUFA, determined using food frequency questionnaire data, was associated with lower risk of RA.[18] However estimated dietary intakes of n-3 PUFA often correlate poorly with in vivo levels of these fatty acids,[19 20] which in turn are also influenced by endogenous processes including elongation, desaturation and β-oxidation. Erythrocyte membranes offer an attractive biomarker of PUFA status in vivo, and reflect dietary intake, uptake and endogenous metabolism over a period of at least 1 month.[19] Recently higher erythrocyte n-3 PUFA levels were found to be associated with a lower risk of RA-related autoantibodies in a population at risk of RA.[21 22] However, there are few data on erythrocyte membrane PUFA and risk of RA. We therefore sought to establish whether there were differences in erythrocyte membrane fatty acid profiles, as a biomarker of PUFA status, between individuals with pre-RA and matched controls in a nested case–control study, and to test the hypothesis that higher levels of long-chain n-3 PUFA are associated with lower risk of RA. As a proinflammatory state may exist prior to the clinical onset of RA, and conceivably might alter PUFA metabolism, we also analysed the relationship between PUFA levels and serum cytokines as a secondary outcome.

METHODS

Study sample

The European Prospective Investigation into Cancer and Nutrition (EPIC) is a multicentre, pan-European prospective cohort study designed to investigate the association between diet and cancer, as well as other diseases, in apparently healthy populations.[23] We undertook a nested case–control study to investigate risk factors for RA, by identifying subjects who developed incident RA after enrolment (referred to here as pre-RA). These pre-RA cases were matched with controls among subjects enrolled in four EPIC cohorts: Naples, Turin and Ragusa in Italy, and Murcia in Spain. Potential RA cases were identified as previously described.[24] In brief, in Murcia, RA cases were identified by linkage with primary healthcare records (International Classification of Primary Care code L88) and prescriptions of disease-modifying antirheumatic drugs, and linkage using the International Classification of Diseases (ICD) codes with hospital discharge (ICD9: 714) and mortality databases (ICD10: M05 and M06). In Naples, RA cases were identified by linkage with hospital discharge databases and information from systematic telephone follow-up of participants. In Turin, RA cases were identified by linkage with hospital discharge databases and a drug prescription database with a disease-specific code. In Ragusa, cases were identified by linkage with hospital discharge databases. Identification of all RA cases was undertaken in 2011. All cases were then subsequently validated by medical record review to confirm a physician diagnosis of RA and to confirm date of diagnosis, as previously described.[24] Subjects with prevalent RA at the time of the blood sample were excluded from the analyses. The EPIC cohorts included in this study recruited subjects between 1992 and 1998.[23]

Three controls were randomly selected from living cohort members and matched for every individual case by age at blood collection (±1 year), sex, centre, date (±2 months) and time (±3 hours) of baseline blood collection, and fasting status at blood collection (<3/3–6/>6 hours). There were erythrocyte samples available for 96 of the 103 pre-RA cases and 258 matched controls.

Data collection

Baseline questionnaires collected detailed data on diet,[23] physical activity, medical history, smoking status, and sociodemographic and lifestyle factors (current and lifetime history). Level of education was defined as having (1) no education, (2) primary school, (3) technical or professional school and (4) higher education (including university degrees). Physical activity was estimated using the Cambridge Physical Activity Index and defined as being either inactive, moderately inactive, moderately active or active.[25] Alcohol intake was estimated as the average alcohol intake (in g/day) using a 24-hour dietary recall. Smoking status was defined as (1) never, (2) former, (3) current smoker and (4) unknown. Anthropometric measures were performed using standardised protocols, and body mass index (BMI) was calculated as weight in kilograms divided by squared height in metres (kg/m^2) and modelled as a continuous variable.

In each centre, blood samples were collected at baseline, transferred to a local laboratory at 5°C–10°C while protected from light and, following processing, erythrocytes were stored in 0.5 mL straws at −196°C in liquid nitrogen. Samples for this study were retrieved and sent on dry ice to a central laboratory at University of Southampton, where they were analysed blinded to case/control status.

Time to diagnosis was defined as the time in years that had elapsed between date of blood sample and date of diagnosis, and was categorised in tertiles (years, median (range): first period: 2.77 (0.82–4.77); second period: 6.70 (4.94–7.99); third period: 10.07 (7.99–15)).

Informed consent was given by all participants.

Erythrocyte fatty acids

Total red cell lipids were extracted with chloroform and methanol (2:1, vol/vol) and dried under nitrogen. The total lipid extracts were dissolved in toluene, and fatty acid methyl esters (FAME) were synthesised by heating the purified lipids at 50°C in the presence of methanol containing 2% (v/v) sulfuric acid. FAMEs were recovered by extraction with hexane and resolved in a BPX-70 fused silica capillary column (32 m×0.25 mm×25 µm; SGE Analytical Science) using an Agilent 6890 gas chromatograph equipped with flame ionisation detection (Agilent Technologies). The relative concentrations (% of total) of individual fatty acids were calculated from the peak area using HP ChemStation (Agilent Technologies).

Long-chain n-3 PUFAs were considered to be EPA, docosapentaenoic acid (22:5 n-3) and DHA. Using the fatty acid measurements, we calculated the sum of the total n-3 PUFA and total n-6 PUFA. Activities of desaturase enzymes, and in particular delta-6 desaturase (D6D), are considered to be rate-limiting steps in the conversion of the precursor n-3 PUFA ALA to the longer chain EPA and DHA, as well as in the parallel metabolism of n-6 PUFA. Activity of desaturase enzymes can be inferred from product to precursor ratios: 20:4 n-6/20:3 n-6 for delta-5 desaturase (D5D) and 18:3 n-6/18:2 n-6 for D6D.[26 27]

Serum cytokines

The following cytokines were measured in serum by multispot assay following the manufacturer's instructions (Meso Scale Diagnostics, Rockville, USA): tumour necrosis factor alpha

(TNFα), interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13 and interferon gamma (IFNγ).

HLA-DRB1 and autoantibodies

Human leucocyte antigen (HLA)-DRB1 gene full exon 2 DNA sequence was established using the Sanger method and sequence-specific oligonucleotide probes (SSOP) hybridisation used for quality assurance. HLA-DRB1 alleles considered to be shared epitope (SE)-positive, a major genetic risk factor for RA, were *0101, *0102, *0401, *0404, *0405, *0408, *0410, *1001, *1402 and *1406. IgM rheumatoid factor (RF) and anticitrullinated protein antibodies (ACPA; measured with an anti-cyclic citrullinated peptide 2 (CCP2) ELISA) were measured in baseline samples as previously described.[24]

Statistical analysis

Fatty acids were categorised in tertiles based on their distribution in the control group, with the lowest category chosen as the referent. Variables with a non-normal distribution were log-transformed for analyses.

Fatty acid levels across the three time periods before RA diagnosis were compared using linear regression. Only one pre-RA subject had a diagnosis <1 year after blood sampling and exclusion of this case did not substantially change the results.

Confirmed pre-RA cases were matched with controls by age at blood collection (±1 year), sex, centre, date (±2 months) and time (±3 hours) of baseline blood collection, and fasting status at blood collection (<3/3–6/>6 hours).

We estimated the association with incident RA of each of the individual n-3 PUFA and n-6 PUFA, total n-3 PUFA, total n-6 PUFA and predefined ratios of PUFA variables.

Conditional logistic regression was conducted to estimate associations of PUFA with incident RA and obtain ORs and 95% CI, adjusting for potential confounders including BMI, waist circumference, education level, physical activity, smoking status and alcohol intake. We performed a complete case analysis. The proportion of subjects with missing data for covariates was <5%. In a sensitivity analysis, the model was further adjusted for the presence of HLA-DRB1 SE. The interaction between SE and LA was tested using multiplicative terms.

As a proinflammatory state may exist prior to the clinical onset of RA, and conceivably might alter PUFA metabolism, the relationship between PUFA levels and serum cytokines was analysed in the pre-RA and control groups separately. We also compared RF-positive pre-RA subjects to RF-negative matched controls using matched conditional logistic regression models, further adjusting for fewer potential confounders (ie, smoking status, alcohol intake, education level and SE) given the smaller sample size.

Given that the cytokine data were overdispersed, negative binomial regression was used to test the relationship between fatty acids (LA, total n-3 and n-6 PUFA, and the n-3:n-6 ratio) and serum cytokine levels, stratified by incident RA, adjusting for age, sex, country of origin, BMI and smoking status. The exponentiated regression coefficients (relative risk (RR)) were interpreted as a ratio of means. The RR indicates a positive or negative association between PUFA and cytokines. We used Stata software for statistical analyses. A P value less than 0.05 was considered as statistically significant. Formal alpha adjustments were not performed.[28–30]

Table 1 Baseline characteristics

	Pre-RA cases	Controls
Characteristics		
N (%)	96 (27)	258 (73)
Age (years), mean (SD)	51 (7.56)	51 (7.30)
Female, n (%)	74 (77)	198 (78)
BMI (kg/m^2), mean (SD)	26.60 (3.70)	26.22 (4.11)
WHR, mean (SD)	0.85 (0.08)	0.84 (0.08)
Smoking status, n (%)		
Never	39 (41)	133 (52)
Former	26 (27)	51 (20)
Current	29 (30)	68 (26)
Unknown	2 (2)	6 (2)
Education, n (%)		
None	18 (19)	30 (12)
Primary school completed	43 (46)	105 (42)
Technical/professional school	10 (11)	26 (10)
Secondary school	17 (18)	47 (19)
Longer education	6 (6)	44 (17)
Physical activity, n (%)		
Inactive	44 (46)	102 (40)
Moderately inactive	29 (30)	85 (33)
Moderately active	11 (11)	44 (17)
Active	10 (10)	21 (8)
Missing	2 (2)	6 (2)
Macronutrients dietary intake, mean (SD)		
Carbohydrate intake*	44.17 (6.81)	44.14 (6.62)
Protein intake*	17.30 (2.37)	17.48 (2.44)
Fat intake*	35.16 (5.19)	34.71 (5.58)
Fibre intake‡	23.01 (7.48)	24.06 (7.87)
Alcohol intake†	22.37 (17.87–28.19)	23.42 (17.98–28.30)
Shared epitope (%)		
No copies	45 (56)	133 (69)
One copy	26 (33)	56 (29)
Two copies	9 (11)	4 (2)
Shared epitope, >1 copies (%)	35 (44)	60 (31)
ACPA-positive (%)¶	23 (24)	9 (3.5)
Rheumatoid factor-positive (%)¶	56 (58)	11 (4)
Time to diagnosis§, mean (SD)	6.71 (3.43)	–

*Macronutrients dietary intake expressed as % of energy.
†Median (IQR).
‡Fibre intake in grams per day.
§Time to diagnosis was defined as time (years) elapsed between date of blood sample and date of diagnosis.
¶Analysis done on samples taken at enrolment.
ACPA, anticitrullinated protein antibodies (determined from baseline samples and not postdiagnosis); BMI, body mass index; RA, rheumatoid arthritis; WHR, waist:hip ratio.

RESULTS

Baseline characteristics of pre-RA cases and controls are shown in table 1. The study sample included 354 individuals, of whom 96 had pre-RA. There were no differences in age, sex, BMI, waist:hip ratio, percentage of energy provided by protein, carbohydrate and fat, and dietary fibre intake between pre-RA cases and controls. Pre-RA individuals were more likely to be former smokers (OR 1.91, 95% CI 1.03 to 3.52) and positive for RF (OR 22.89; 95% CI 9.83 to 53.33) and ACPA (OR 5.22; 95% CI 1.74 to 15.69).

Table 2 Risk of RA by PUFA tertiles in the EPIC study

	Tertiles of PUFA			
	1 (reference)	2	3	
	OR (95% CI)	OR (95% CI)	OR (95% CI)	P for trend*
PUFA				
18:2 n-6† (LA)	10.73 (9.69 to 11.61)	14.15 (13.33 to 14.74)	16.73 (15.93 to 17.92)	
Model 1	1	0.66 (0.33 to 1.31)	0.37 (0.16 to 0.84)	0.018
Model 2	1	0.52 (0.24 to 1.11)	0.29 (0.12 to 0.75)	0.010
18:3 n-6† (GLA)	0.004 (0.003 to 0.004)	0.006 (0.005 to 0.006)	0.009 (0.008 to 0.011)	
Model 1	1	1.64 (0.86 to 3.13)	1.16 (0.59 to 2.28)	0.782
Model 2	1	1.73 (0.85 to 3.51)	1.18 (0.57 to 2.44)	0.754
18:3 n-3† (ALA)	0.10 (0.06 to 0.11)	0.20 (0.16 to 0.24)	0.48 (0.34 to 0.68)	
Model 1	1	1.33 (0.72 to 2.45)	0.97 (0.52 to 1.82)	0.928
Model 2	1	1.47 (0.74 to 2.93)	1.02 (0.52 to 1.99)	0.981
20:2 n-6†	0.29 (0.25 to 0.31)	0.36 (0.34 to 0.37)	0.45 (0.41 to 0.50)	
Model 1	1	0.93 (0.49 to 1.76)	0.76 (0.40 to 1.44)	0.393
Model 2	1	1.13 (0.57 to 2.25)	0.80 (0.39 to 1.63)	0.538
20:3 n-6†	1.55 (1.38 to 1.69)	1.97 (1.87 to 2.05)	2.51 (2.34 to 2.81)	
Model 1	1	1.23 (0.66 to 2.30)	1.45 (0.78 to 2.68)	0.238
Model 2	1	1.38 (0.69 to 2.74)	1.54 (0.77 to 3.09)	0.218
20:4 n-6† (AA)	7.20 (6.47 to 7.95)	9.53 (9.06 to 9.97)	12.14 (11.23 to 13.16)	
Model 1	1	0.68 (0.36 to 1.28)	1.04 (0.57 to 1.90)	0.777
Model 2	1	0.671 (0.337 to 1.337)	0.969 (0.500 to 1.879)	0.994
20:4 n-3†	0.03 (0.02 to 0.03)	0.05 (0.04 to 0.05)	0.08 (0.07 to 0.09)	
Model 1	1	2.01 (1.04 to 3.86)	1.78 (0.89 to 3.54)	0.111
Model 2	1	1.82 (0.87 to 3.77)	1.82 (0.86 to 3.83)	0.138
20:5 n-3 (EPA)†	0.43 (0.32 to 0.48)	0.68 (0.63 to 0.76)	1.07 (0.93 to 1.27)	
Model 1	1	1.85 (0.98 to 3.49)	1.06 (0.51 to 2.21)	0.977
Model 2	1	1.92 (0.93 to 3.88)	1.26 (0.55 to 2.88)	0.661
22:4 n-6†	0.76 (0.55 to 1.05)	0.64 (0.49 to 0.89)	0.68 (0.49 to 0.88)	
Model 1	1	1.49 (0.81 to 2.76)	1.646 (0.814 to 3.329)	0.167
Model 2	1	1.38 (0.72 to 2.67)	1.59 (0.75 to 3.33)	0.225
22:5 n-3†	0.01 (0.009 to 0.02)	0.01 (0.008 to 0.01)	0.01 (0.01 to 0.02)	
Model 1	1	1.01 (0.56 to 1.82)	1.66 (0.86 to 3.19)	0.157
Model 2	1	1.04 (0.55 to 1.99)	1.70 (0.84 to 3.45)	0.150
22:6 n-3 (DHA)†	1.58 (1.37 to 1.890)	1.75 (1.53 to 2.03)	1.97 (1.66 to 2.24)	
Model 1	1	1.92 (0.98 to 3.75)	1.58 (0.79 to 3.12)	0.226
Model 2	1	1.80 (0.87 to 3.73)	1.54 (0.73 to 3.26)	0.301
Total n-3 PUFA†	4.89 (4.49 to 5.33)	6.09 (5.79 to 6.35)	7.33 (6.93 to 7.95)	
Model 1	1	2.05 (1.10 to 3.80)	1.44 (0.72 to 2.86)	0.368
Model 2	1	1.82 (0.90 to 3.67)	1.64 (0.77 to 3.52)	0.252
Total n-6 PUFA†	22.26 (20.96 to 23.44)	25.82 (25.17 to 26.61)	29.88 (28.65 to 31.61)	
Model 1	1	0.80 (0.43 to 1.47)	0.63 (0.32 to 1.24)	0.181
Model 2	1	0.78 (0.39 to 1.54)	0.58 (0.28 to 1.21)	0.149
n-3:n-6 ratio†	0.18 (0.16 to 0.19)	0.23 (0.21 to 0.25)	9.29 (0.27–0.32)	
Model 1	1	1.80 (0.97 to 3.35)	1.56 (0.80 to 3.05)	0.216
Model 2	1	1.61 (0.81 to 3.19)	1.86 (0.88 to 3.95)	0.108
D6D†	0.0002 (0.0002 to 0.0003)	0.0004 (0.0004 to 0.0005)	0.0008 (0.0006 to 0.001)	
Model 1	1	1.59 (0.84 to 2.99)	1.42 (0.66 to 3.04)	0.360
Model 2	1	1.57 (0.79 to 3.12)	1.52 (0.66 to 3.47)	0.306
D5D†	3.37 (2.92 to 3.75)	4.68 (4.30 to 5.04)	6.69 (6.01 to 7.96)	
Model 1	1	0.64 (0.35 to 1.17)	0.81 (0.43 to 1.51)	0.473
Model 2	1	0.64 (0.33 to 1.25)	0.82 (0.42 to 1.62)	0.556
LC n-3 PUFA†	4.60 (4.01 to 4.89)	5.78 (5.48 to 6.04)	6.94 (6.63 to 7.58)	
Model 1	1	1.94 (1.04 to 3.62)	1.60 (0.82 to 3.12)	0.036
Model 2	1	1.63 (0.81 to 3.25)	1.66 (0.80 to 3.45)	0.197

Model 1: conditional logistic regression model matched by age at blood collection (±1 year), sex, centre, date (±2 months) and time (±3 hours) of baseline blood collection, and fasting status at blood collection (<3/3–6/>6 hours). There were erythrocyte samples available for 96 of the 103 pre-RA cases and 258 matched controls.
Model 2 is model 1 with further adjustment for BMI, waist circumference, education level, physical activity, smoking status and alcohol intake.
*The significance of linear trends across tertiles was tested with linear regression models.
†Fatty acids are expressed as a percentage, median (IQR) of total erythrocyte membrane fatty acids.
AA, arachidonic acid; ALA, alpha -linolenic acid ; BMI, body mass index; D5D, delta-5 desaturase activity inferred from product to precursor ratios; D6D, delta-6 desaturase activity inferred from product to precursor ratios; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; EPIC, European Prospective Investigation into Cancer and Nutrition; GLA, G amma -linolenic acid; LA, linoleic acid; LC PUFA: total longer chain n-3 PUFA; PUFA, polyunsaturated fatty acids; RA, rheumatoid arthritis.

Table 3Polyunsaturated fatty acids levels across three time periods (tertiles) before the diagnosis of RA

	Pre-RA time periods			
	1	2	3	P value
Time*	2.77 (0.82–4.78)	6.70 (4.94–7.99)	10.07 (7.99–15)	
PUFA levels†				
Linoleic acid	13.00 (3.19)	13.43 (2.95)	14.45 (3.21)	0.06
20:3 n-6	2.18 (0.53)	2.12 (0.51)	1.89 (0.39)	0.02
22:5 n-3	1.95 (0.42)	1.85 (0.43)	1.71 (0.42)	0.02

*Time in years, median (range). Median time to diagnosis, defined as time in years elapsed between date of blood sample and date of diagnosis, was 6.7 years (range 0.8–15). For this analysis the time to diagnosis was categorised into three periods based on tertiles (years, median (range): first period: 2.77 (0.82–4.77); second period: 6.70 (4.94–7.99); and third period: 10.07 (7.99–15)).
†Mean (±SD).
PUFA, fatty acid; RA, rheumatoid arthritis.

The relationship between tertiles of n-3 and n-6 PUFA and risk of RA is shown in table 2. A significant inverse association was observed with the n-6 PUFA LA (highest tertile: OR 0.29; 95% CI 0.12 to 0.75; P for trend 0.01) and pre-RA in the fully adjusted model. However, no association was observed with any of the individual n-3 PUFA, total n-3 PUFA or total long-chain n-3 PUFA, or with the total n-3:n-6 ratio. In a sensitivity analysis, a model with further adjustment for the presence of the HLA-DRB1 SE confirmed the significant inverse association with the n-6 PUFA LA and pre-RA (second tertile: OR 0.32; 95% CI 0.13 to 0.79; highest tertile: 0.21; 95% CI 0.07 to 0.67; P for trend 0.008). No interaction was observed between LA and the presence of the HLA-DRB1 SE (1 or 2 copies) in relation to risk for RA (P=0.68).

Longitudinal samples were not available, but we did compare pre-RA subjects across different time periods before diagnosis, and found a significant difference in the median PUFA membrane content of 20:3 n-6 and 22:5 n-3 with increasing levels closer to diagnosis (table 3). Compared with the period of time immediately before the diagnosis (period 1), 20:3 n-6 and 22:5 n-3 were significantly lower in the period of time furthest away from diagnosis (period 3) (β −0.14, 95% CI −0.25 to −0.02, P=0.02; and β −0.25, 95% CI − 0.46 to −0.03, P=0.02, for 20:3 n-6 and 22:5 n-3, respectively). Conversely there was a trend for decreasing levels of LA closer to diagnosis (data for other PUFA not shown).

Because final autoantibody status was not known for all subjects, we did not compare the effects of PUFA on risk for autoantibody-positive versus autoantibody-negative RA. However, when restricting the analysis to pre-RA subjects who had documented RF postdiagnosis or a positive RF baseline sample (n=56 (58%)), compared with RF-negative matched controls (n=245 (96%)), an inverse association was also observed with LA for RF-positive pre-RA (lowest tertile: OR 1 (reference); second tertile: OR 0.18; 95% CI 0.03 to 0.96; highest tertile: OR 0.13; 95% CI 0.02 to 0.90; P for trend=0.04) in a multivariable-adjusted model.

The relationships between levels of LA, total n-3 and n-6 PUFA and the n-3:n-6 ratio and serum cytokines are displayed in online supplementary table 1 for the control population and online supplementary table 2 for the pre-RA population. In the control population, LA was positively associated with levels of TNFα and IL-6 but negatively associated with IL-4, IL-5, IL-10, IL-12, IL-13 and IFNγ in fully adjusted models. In the pre-RA population, LA remained positively associated with TNFα and also IL-1, but was now negatively associated with IL-6. Negative associations between LA and IL-5, IL-12 and IL-13 persisted in the pre-RA group. The n-3:n-6 ratio was inversely associated with TNFα and IL-1 in both groups.

DISCUSSION

We observed that erythrocyte membrane levels of the n-6 PUFA LA were inversely proportional to the risk of developing RA, whereas no protective effect was observed with n-3 PUFA. Previous studies have reported that a higher estimated intake of n-3 fatty acids may be protective against the development of RA in Swedish women,[18] and that erythrocyte n-3 levels were inversely related to the risk of RA-related autoimmunity in a population at risk of RA.[21 22] This discrepancy with our findings may first be explained by n-3 bioavailability, since previous research has shown lower long-chain n-3 PUFA blood levels in southern European populations compared with those from Scandinavia,[31] despite a similar estimated dietary intake of seafood n-3 PUFA.[32] In line with this, we observed mean erythrocyte levels of n-3 fatty acids in the control group that were lower than those observed in northern Sweden, with levels of EPA almost half (0.75% vs 1.4%).[33 34] Second, Gan *et al*[21] found that higher erythrocyte n-3 fatty acid levels were protective against RA-related autoimmunity only in patients who were HLA-DRB1 SE-positive, and hypothesised that this might be due to the ability of the n-3 PUFA DHA to alter lipid rafts and so influence antigen presentation by HLA molecules or regulatory T cell function. Notably, the prevalence of the SE is considerably higher in northern European RA populations compared with those from southern Europe (approaching 80% in some population-based studies[35] vs 44% in the pre-RA group studied here).

We did, however, observe that higher levels of the n-6 PUFA LA were associated with lower risk for RA. LA was also negatively associated with RF positive RA, which overlaps considerably with the presence of ACPA, but no interaction was observed with the SE. A key question is whether LA is protective against RA, or whether lower LA levels in the pre-RA group are secondary to metabolic and inflammatory changes occurring before the clinical onset of RA. Interestingly, previous small studies in established RA have identified lower plasma phospholipid and adipose tissue LA relative to controls.[36 37] This was thought to be reverse causation, with a metabolic state in RA driving increased desaturase enzyme activity for which LA is a substrate. Indeed, we have previously suggested that D5D activity may be reduced following treatment with anti-TNF.[38] In this cohort we did not find D5D or D6D product to precursor ratios to be associated with risk of RA, or to vary with time to diagnosis, but we did observe a non-statistically significant trend for lower LA levels in subjects closest in time to RA diagnosis. Furthermore, we found increasing levels of 20:3 n-6 and 22:5 n-3 in subjects closest to diagnosis, compatible with increased production of downstream products from the metabolism of n-6 LA and n-3 ALA. Other published reports point to metabolic alterations occurring in the years before clinical onset of RA, including a reduction in total cholesterol and low-density lipoprotein and alterations in tryptophan metabolism.[39 40]

Certainly reverse causation as an explanation of our LA findings would be more compatible with the widespread conception that n-6 PUFAs are proinflammatory. However the evidence to support this conception in humans in vivo is sparse.[41] Indeed, data from non-RA population-based studies have suggested that intake of n-6 PUFA is inversely associated with C-reactive protein (CRP) and certain proinflammatory cytokines.[42–45] Furthermore, n-6 PUFA intake has been positively associated

with levels of the anti-inflammatory cytokine transforming growth factor beta (TGFβ),[42] and n-6 PUFA supplementation in healthy volunteers increases TGFβ production by peripheral blood mononuclear cells ex vivo.[46] The n-6 PUFA arachidonic acid gives rise to prostaglandins and leucotrienes that are proinflammatory in a context-dependent fashion, but n-6 PUFA can also give rise to potent anti-inflammatory mediators such as lipoxins,[47] 13-hydroxyoctadecadienoic acid[48] and nitrated-LA.[49] Higher doses of the longer chain n-6 PUFA gamma-linolenic acid were found in a small study to improve the signs and symptoms of RA.[50]

Regarding other health outcomes, a meta-analysis of prospective studies on fatty acids and risk of coronary heart disease (CHD) observed an independent inverse association between LA and CHD risk (RR of CHD: 0.91; 95% CI 0.84 to 0.98). Other fatty acids were not associated with CHD.[51] More recently, the Cardiovascular Health Study, a community-based US cohort of 2792 participants aged 65 years and older free of CVD at baseline, observed that high circulating LA, but not other n-6 PUFA, was inversely associated with total and CHD mortality.[52] A recent large cohort study including over 27 000 participants in the EPIC-InterAct study across European countries observed that LA is inversely associated with type 2 diabetes (T2D). The results also suggested an inverse association of ALA but no convincing association of marine-derived n-3 PUFA with T2D.[27] Also a recent large pooled analysis of individual-level data for nearly 40 000 adults from 20 prospective studies has shown that higher levels of LA biomarkers were independently associated with a lower risk of T2D.[53]

Given our findings on LA and risk of RA, we explored the relationship of LA and serum cytokines in this population. The observed positive association between LA and TNFα and IL-6 in our control group is consistent with the hypothesis that high n-6 PUFA and LA are proinflammatory, and the n-3:n-6 ratio was inversely associated with TNFα and IL-1 levels in both the controls and the pre-RA group. Also in support of inflammation driving a reduction in LA is the shift from a positive association between LA and IL-6 in the control population, to a negative association in the pre-RA group, although interestingly both TNFα and IL-1 remain positively associated with LA in pre-RA. Also consistent with prevailing notions is our finding of a negative association between LA and Th2 cytokines IL-4, IL-5 and IL-13, and also IL-10, which is produced by monocytes and to a lesser extent Th2 cells, and has well-established anti-inflammatory functions. However LA was also negatively associated with IL-12, which induces Th1 responses, and also the Th1 cytokine IFNγ, implying more generally downregulated T cell function. The important role of T cells in the pathogenesis of RA, and the observation that high levels of Th2 cytokines have been shown in the earliest phase of the disease,[54] could support the hypothesis of high LA levels being protective in this population. Furthermore, polymorphisms in the fatty acid desaturase (FADS) genes, which encode the rate-limiting enzymes for the biosynthesis of longer chain n-6 and n-3 PUFA, have been identified as being risk factors for RA, suggesting that PUFA status may contribute to RA pathogenesis.[55]

The strengths of the present study are the large number of matched controls and pre-RA cases from a population-based cohort and measurement of erythrocyte PUFA and cytokines in samples taken prior to clinical onset of RA. The measure of objective biomarkers allowed investigation of individual PUFA while avoiding potential errors and recall bias related to self-reported dietary intake. Indeed total erythrocyte n-3 PUFA status did not correlate with fish intake in this cohort (data not shown). We also took careful control of important potential confounding factors. However, residual and unmeasured confounding remains an important limitation; this may be particularly relevant to studies of specific nutrients, which themselves are part of more complex dietary intake.

The analyses of associations between PUFA and incident RA involved multiple tests of significance. A potential limitation relates to multiple testing, because multiple comparisons are associated with an increased chance for a type I error. However, the utility of formal adjustments for multiple comparisons in an epidemiological context has been questioned.[28–30] We therefore chose not to perform any formal alpha adjustments in the present study and acknowledge the need to consider with caution the statistical significance of such associations.

In conclusion, we found erythrocyte levels of LA, the major dietary n-6 PUFA, were inversely associated with risk of pre-RA. Other n-6 PUFA and n-3 PUFA were not significantly associated with risk of RA. Further work is warranted to replicate these findings and to investigate if LA is protective against RA development or if lower levels are secondary to metabolic and inflammatory changes occurring before the clinical onset of RA.

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REFERENCES

1 Frisell T, Saevarsdottir S, Askling J. Family history of rheumatoid arthritis: an old concept with new developments. *Nat Rev Rheumatol* 2016;12:335–43.
2 Karlson EW, Deane K. Environmental and gene-environment interactions and risk of rheumatoid arthritis. *Rheum Dis Clin North Am* 2012;38:405–26.
3 Fisher BA, Bang SY, Chowdhury M, *et al*. Smoking, the HLA-DRB1 shared epitope and ACPA fine-specificity in Koreans with rheumatoid arthritis: evidence for more than one pathogenic pathway linking smoking to disease. *Ann Rheum Dis* 2014;73:741–7.
4 Calder PC. Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology? *Br J Clin Pharmacol* 2013;75:645–62.
5 Williams CM, Burdge G. Long-chain n-3 PUFA: plant v. marine sources. *Proc Nutr Soc* 2006;65:42–50.
6 Arterburn LM, Hall EB, Oken H. Distribution, interconversion, and dose response of n-3 fatty acids in humans. *Am J Clin Nutr* 2006;83:1467s–76.
7 Harbige LS. Fatty acids, the immune response, and autoimmunity: a question of n-6 essentiality and the balance between n-6 and n-3. *Lipids* 2003;38:323–41.
8 Ierna M, Kerr A, Scales H, *et al*. Supplementation of diet with krill oil protects against experimental rheumatoid arthritis. *BMC Musculoskelet Disord* 2010;11:136.
9 Leslie CA, Gonnerman WA, Ullman MD, *et al*. Dietary fish oil modulates macrophage fatty acids and decreases arthritis susceptibility in mice. *J Exp Med* 1985;162:1336–49.
10 Miles EA, Calder PC. Influence of marine n-3 polyunsaturated fatty acids on immune function and a systematic review of their effects on clinical outcomes in rheumatoid arthritis. *Br J Nutr* 2012;107(Suppl 2):S171–84.
11 Proudman SM, James MJ, Spargo LD, *et al*. Fish oil in recent onset rheumatoid arthritis: a randomised, double-blind controlled trial within algorithm-based drug use. *Ann Rheum Dis* 2015;74:89–95.
12 Kremer JM. n-3 fatty acid supplements in rheumatoid arthritis. *Am J Clin Nutr* 2000;71():349s–51.
13 Benito-Garcia E, Feskanich D, Hu FB, Fb H, *et al*. Protein, iron, and meat consumption and risk for rheumatoid arthritis: a prospective cohort study. *Arthritis Res Ther* 2007;9:R16.
14 Di Giuseppe D, Crippa A, Orsini N, *et al*. Fish consumption and risk of rheumatoid arthritis: a dose-response meta-analysis. *Arthritis Res Ther* 2014;16:446.
15 Pedersen M, Stripp C, Klarlund M, *et al*. Diet and risk of rheumatoid arthritis in a prospective cohort. *J Rheumatol* 2005;32:1249–52.
16 Rosell M, Wesley AM, Rydin K, *et al*. Dietary fish and fish oil and the risk of rheumatoid arthritis. *Epidemiology* 2009;20:896–901.
17 Shapiro JA, Koepsell TD, Voigt LF, *et al*. Diet and rheumatoid arthritis in women: a possible protective effect of fish consumption. *Epidemiology* 1996;7:256–63.
18 Di Giuseppe D, Wallin A, Bottai M, *et al*. Long-term intake of dietary long-chain n-3 polyunsaturated fatty acids and risk of rheumatoid arthritis: a prospective cohort study of women. *Ann Rheum Dis* 2014;73:1949–53.
19 Arab L. Biomarkers of fat and fatty acid intake. *J Nutr* 2003;133(Suppl 3):925S–32.
20 Astorg P, Bertrais S, Laporte F, *et al*. Plasma n-6 and n-3 polyunsaturated fatty acids as biomarkers of their dietary intakes: a cross-sectional study within a cohort of middle-aged French men and women. *Eur J Clin Nutr* 2008;62:1155–61.
21 Gan RW, Demoruelle MK, Deane KD, *et al*. Omega-3 fatty acids are associated with a lower prevalence of autoantibodies in shared epitope-positive subjects at risk for rheumatoid arthritis. *Ann Rheum Dis* 2017;76.
22 Gan RW, Young KA, Zerbe GO, *et al*. Lower omega-3 fatty acids are associated with the presence of anti-cyclic citrullinated peptide autoantibodies in a population at risk for future rheumatoid arthritis: a nested case-control study. *Rheumatology* 2016;55:367–76.
23 Riboli E, Hunt KJ, Slimani N, *et al*. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr* 2002;5:1113–24.
24 Fisher BA, Cartwright AJ, Quirke AM, *et al*. Smoking, Porphyromonas gingivalis and the immune response to citrullinated autoantigens before the clinical onset of rheumatoid arthritis in a Southern European nested case-control study. *BMC Musculoskelet Disord* 2015;16:331.
25 Peters T, Brage S, Westgate K, *et al*. Validity of a short questionnaire to assess physical activity in 10 European countries. *Eur J Epidemiol* 2012;27:15–25.
26 Tosi F, Sartori F, Guarini P, *et al*. Delta-5 and delta-6 desaturases: crucial enzymes in polyunsaturated fatty acid-related pathways with pleiotropic influences in health and disease. *Adv Exp Med Biol* 2014;824:61–81.
27 Forouhi NG, Imamura F, Sharp SJ, *et al*. Association of Plasma Phospholipid n-3 and n-6 Polyunsaturated Fatty Acids with Type 2 Diabetes: The EPIC-InterAct Case-Cohort Study. *PLoS Med* 2016;13:e1002094.
28 Perneger TV. What's wrong with Bonferroni adjustments. *BMJ* 1998;316:1236–8.
29 Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiology* 1990;1:43–6.
30 Savitz DA, Olshan AF. Multiple comparisons and related issues in the interpretation of epidemiologic data. *Am J Epidemiol* 1995;142:904–8.
31 Stark KD, Van Elswyk ME, Higgins MR, *et al*. Global survey of the omega-3 fatty acids, docosahexaenoic acid and eicosapentaenoic acid in the blood stream of healthy adults. *Prog Lipid Res* 2016;63:132–52.
32 Micha R, Khatibzadeh S, Shi P, *et al*. Global, regional, and national consumption levels of dietary fats and oils in 1990 and 2010: a systematic analysis including 266 country-specific nutrition surveys. *BMJ* 2014;348:g2272.
33 Krachler B, Norberg M, Eriksson JW, *et al*. Fatty acid profile of the erythrocyte membrane preceding development of Type 2 diabetes mellitus. *Nutr Metab Cardiovasc Dis* 2008;18:503–10.
34 Wennberg M, Vessby B, Johansson I. Evaluation of relative intake of fatty acids according to the Northern Sweden FFQ with fatty acid levels in erythrocyte membranes as biomarkers. *Public Health Nutr* 2009;12:1477–84.
35 Mahdi H, Fisher BA, Källberg H, *et al*. Specific interaction between genotype, smoking and autoimmunity to citrullinated alpha-enolase in the etiology of rheumatoid arthritis. *Nat Genet* 2009;41:1319–24.
36 Bruderlein H, Daniel R, Boismenu D, *et al*. Fatty acid profiles of serum phospholipids in patients suffering rheumatoid arthritis. *Prog Lipid Res* 1981;20:625–31.
37 Jacobsson L, Lindgärde F, Manthorpe R, *et al*. Correlation of fatty acid composition of adipose tissue lipids and serum phosphatidylcholine and serum concentrations of micronutrients with disease duration in rheumatoid arthritis. *Ann Rheum Dis* 1990;49:901–5.
38 Jeffery L, Fisk HL, Calder PC, *et al*. Plasma Levels of Eicosapentaenoic Acid Are Associated with Anti-TNF Responsiveness in Rheumatoid Arthritis and Inhibit the Etanercept-driven Rise in Th17 Cell Differentiation in Vitro. *J Rheumatol* 2017;44:748–56.
39 Myasoedova E, Crowson CS, Kremers HM, *et al*. Total cholesterol and LDL levels decrease before rheumatoid arthritis. *Ann Rheum Dis* 2010;69:1310–4.
40 Surowiec I, Ärlestig L, Rantapää-Dahlqvist S, *et al*. Metabolite and Lipid Profiling of Biobank Plasma Samples Collected Prior to Onset of Rheumatoid Arthritis. *PLoS One* 2016;11:e0164196.
41 Fritsche KL. Too much linoleic acid promotes inflammation-doesn't it? *Prostaglandins Leukot Essent Fatty Acids* 2008;79:173–5.
42 Ferrucci L, Cherubini A, Bandinelli S, *et al*. Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers. *J Clin Endocrinol Metab* 2006;91:439–46.
43 Julia C, Touvier M, Meunier N, *et al*. Intakes of PUFAs were inversely associated with plasma C-reactive protein 12 years later in a middle-aged population with vitamin E intake as an effect modifier. *J Nutr* 2013;143:1760–6.
44 Kalogeropoulos N, Panagiotakos DB, Pitsavos C, *et al*. Unsaturated fatty acids are inversely associated and n-6/n-3 ratios are positively related to inflammation and coagulation markers in plasma of apparently healthy adults. *Clin Chim Acta* 2010;411:584–91.
45 Poudel-Tandukar K, Nanri A, Matsushita Y, *et al*. Dietary intakes of alpha-linolenic and linoleic acids are inversely associated with serum C-reactive protein levels among Japanese men. *Nutr Res* 2009;29:363–70.
46 Fisher BA, Harbige LS. Effect of omega-6 lipid-rich borage oil feeding on immune function in healthy volunteers. *Biochem Soc Trans* 1997;25:343S.
47 Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nat Rev Immunol* 2008;8:349–61.
48 Wittwer J, Hersberger M. The two faces of the 15-lipoxygenase in atherosclerosis. *Prostaglandins Leukot Essent Fatty Acids* 2007;77:67–77.
49 Baker PR, Lin Y, Schopfer FJ, *et al*. Fatty acid transduction of nitric oxide signaling: multiple nitrated unsaturated fatty acid derivatives exist in human blood and urine and serve as endogenous peroxisome proliferator-activated receptor ligands. *The Journal of biological chemistry* 2005;280:42464–75.
50 Zurier RB, Rossetti RG, Jacobson EW, *et al*. gamma-Linolenic acid treatment of rheumatoid arthritis. A randomized, placebo-controlled trial. *Arthritis Rheum* 1996;39:1808–17.
51 de Goede J, Verschuren WM, Boer JM, *et al*. N-6 and N-3 fatty acid cholesteryl esters in relation to fatal CHD in a Dutch adult population: a nested case-control study and meta-analysis. *PLoS One* 2013;8:e59408.
52 Wu JH, Lemaitre RN, King IB, *et al*. Circulating omega-6 polyunsaturated fatty acids and total and cause-specific mortality: the Cardiovascular Health Study. *Circulation* 2014;130:1245–53.
53 Jhy W, Marklund M, Imamura F, *et al*. Omega-6 fatty acid biomarkers and incident type 2 diabetes: pooled analysis of individual-level data for 39 740 adults from 20 prospective cohort studies. *The lancet Diabetes & endocrinology* 2017;5:965–74.
54 Raza K, Falciani F, Curnow SJ, *et al*. Early rheumatoid arthritis is characterized by a distinct and transient synovial fluid cytokine profile of T cell and stromal cell origin. *Arthritis Res Ther* 2005;7:R784–95.
55 Okada Y, Wu D, Trynka G, *et al*. Genetics of rheumatoid arthritis contributes to biology and drug discovery. *Nature* 2014;506:376–81.
56 de Pablo P, Romaguera D, Fisk H, *et al*. High Erythrocyte Levels of the n-6 Polyunsaturated Fatty Acid Linoleic Acid Are Associated with Lower Risk of Subsequent Rheumatoid Arthritis in a Southern European Nested Case-Control Study [abstract]. *Arthritis Rheumatol* 2017;69.

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EXTENDED REPORT

Lipid profile and effect of statin treatment in pooled phase II and phase III baricitinib studies

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ABSTRACT

Objectives Lipid profiles are altered by active disease in patients with rheumatoid arthritis (RA) and may be further modified by treatment with Janus kinase inhibitors and other disease-modifying antirheumatic drugs.

Methods Lipid data were analysed from phase II and III studies of 4 mg (n=997) and 2 mg (n=479) oral baricitinib administered once daily in patients with moderate-to-severe active RA. Lipoprotein particle size and number and GlycA were evaluated with nuclear magnetic resonance in one phase III study. The effect of statin therapy on lipid levels was evaluated in patients on statins at baseline and in patients who initiated statins during the study.

Results Treatment with baricitinib was associated with increased levels of total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides, but no significant change in LDL-C:HDL-C ratio. Lipid levels plateaued after 12 weeks of treatment. Baricitinib treatment increased large LDL and decreased small, dense LDL particle numbers and GlycA. Lipid changes from baseline were not significantly different between baseline statin users and non-users. In patients who initiated statin therapy during the study, LDL-C, triglycerides (baricitinib 4 mg only) and apolipoprotein B decreased to pre-baricitinib levels; HDL-C and apolipoprotein A-I levels remained elevated.

Conclusions Baricitinib was associated with increased LDL-C, HDL-C and triglyceride levels, but did not alter the LDL-C:HDL-C ratio. Evaluation of cardiovascular event rates during long-term treatment is warranted to further characterise these findings and their possible clinical implications.

Trial registration number NCT00902486, NCT01469013, NCT01185353, NCT01721044, NCT01721057, NCT01711359, NCT01710358, NCT01885078.

INTRODUCTION

Baricitinib, an oral selective inhibitor of Janus kinase (JAK) 1 and JAK 2,[1] is approved in the European Union and Japan for the treatment of moderate-to-severe active rheumatoid arthritis (RA) in adults. Baricitinib improved signs and symptoms of RA in phase III, placebo and active-controlled studies in patients with active RA who were naïve to conventional synthetic disease-modifying antirheumatic drugs (csDMARD; RA-BEGIN),[2] or had an inadequate response (IR) to previous treatment with methotrexate (MTX; RA-BEAM),[3] csDMARDs (RA-BUILD)[4] and biological DMARDs (RA-BEACON).[5]

In RA, the proinflammatory state in untreated patients is associated with a decrease in total cholesterol, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C)[6]; anti-inflammatory therapies have been shown to increase these lipid levels.[7–11] Increases in lipids persisted in a phase II study of baricitinib.[12] The increase in LDL-C was associated with an increase in large and a decrease in small LDL, without any increase in LDL particle number. The increase in HDL-C was associated with an increase in HDL particle number across all particle sizes.[12] This LDL and HDL particle profile has been associated with reduced atherogenic risk.[13]

This analysis assessed the effects of baricitinib on the lipid profile, lipoprotein particle size and number, and GlycA. GlycA, a measure of glycosylated acute phase proteins, is an emerging inflammatory marker that may be useful for assessing disease activity and is associated with subclinical cardiovascular disease in patients with RA.[14–19] In addition, the effect of statin therapy on these biomarkers was evaluated. The impact of lipid alteration on cardiovascular risk, changes in risk scores and association between LDL-C change and major adverse cardiovascular events (MACE) were also assessed.

METHODS

Study design and patients

Data were included from seven randomised clinical studies: three phase II (JADA, JADC and JADN) and four phase III studies (RA-BEGIN, RA-BEAM, RA-BUILD and RA-BEACON), including data from the long-term extension (LTE) (RA-BEYOND; data through 1 January 2016). All patients completing phase III studies or JADA were eligible to enter RA-BEYOND. The designs for each study have been previously described[2–5 20–23] and are summarised in online supplementary table S1.

Each study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice Guidelines. All patients provided written informed consent. The studies were designed by the sponsors, Eli Lilly and Company and Incyte,

with input from an academic advisory board in which non-Lilly authors of this manuscript participated. All authors participated in the preparation and review of this manuscript and approved the final version.

Data sets

Three data sets are defined: the six-study placebo-controlled set, the long-term baricitinib cohort and the all-baricitinib RA-MACE set. The six-study set included all phase II/III studies with placebo and baricitinib 4 mg treatment arms (JADA, JADC and JADN; and RA-BEAM, RA-BUILD and RA-BEACON; JADA, JADN, RA-BUILD and RA-BEACON also had a baricitinib 2 mg treatment arm (online supplementary table S1)). The six-study set included data up to week 24 that were censored at rescue or the end of the placebo-controlled period. The long-term baricitinib cohort comprised patients randomised to baricitinib 4 mg in RA-BEAM, RA-BUILD and RA-BEACON, and patients randomised to baricitinib 2 mg in RA-BUILD and RA-BEACON, including data from RA-BEYOND, with data censored at dose change or rescue in the LTE. The all-baricitinib RA-MACE set included all patients from the phase III studies, including patients in the LTE who received at least one baricitinib dose.

Lipid profile

Standard lipid panel (total cholesterol, LDL-C, HDL-C and triglycerides) was assessed for all studies; apolipoprotein A-I and apolipoprotein B for JADA and all phase III studies including the LTE; and nuclear magnetic resonance (NMR) spectroscopy panel (lipoprotein particles and GlycA) for JADA and RA-BEAM.

Serum samples for lipid profile were collected in a fasting state at baseline and weeks 12 and 24 after randomisation; results are reported from pooled data of the six-study placebo-controlled set. In the phase III studies, LDL-C was quantified by direct method; in the phase II studies, LDL-C was calculated using the Friedewald equation, unless triglycerides were >4.52 mmol/L, then LDL-C was quantified by direct method. Lipid treatment-emergent highest values are reported based on criteria from the National Lipids Association Guidelines.[24] Lipid profile up to 104 weeks was evaluated using the long-term baricitinib cohort.

NMR analysis

Determination of lipoprotein particle subfractions by NMR was assessed in RA-BEAM, with fasting serum samples collected at baseline and week 12 (LipoScience, now LabCorp, Morrisville, NC). Diameter ranges (nm) were 21.2–23 for large LDL, 18–21.2 for small LDL, 8.8–13 for large HDL, 8.2–8.8 for medium HDL and 7.3–8.2 for small HDL, as determined by the LP2 algorithm.[25] Apolipoprotein A-I and apolipoprotein B serum samples were analysed at baseline and weeks 4 and 12 and were quantified using conventional ELISA (Pacific Biomarkers, Seattle, WA). GlycA levels were quantified by NMR in JADA and RA-BEAM at baseline and weeks 12 and 24 as previously described.[14]

Statin use

Lipid effects by baseline statin use were evaluated in the six-study placebo-controlled set; data are presented for the placebo and baricitinib combined 2/4 mg groups. Lipid effects for patients who initiated statins during studies were evaluated in the six-study set for patients randomised to placebo and in the long-term cohort for patients randomised to baricitinib (2 and 4 mg groups presented separately).

Cardiovascular risk assessment

To evaluate the risk of cardiovascular disease, the Framingham Risk Score[26] and Reynolds Risk Score[27 28] were assessed at baseline and week 24 in the phase III studies. Relationships were explored in the all-baricitinib RA-MACE set between change in LDL-C and MACE, a composite measure that includes cardiovascular death, stroke and myocardial infarction. An independent, external clinical endpoint committee adjudicated all death and potential cardiovascular events.

Statistical analysis

Conventional lipid profiles including apolipoprotein A-I and apolipoprotein B were assessed using analysis of covariance (ANCOVA), adjusting for baseline value, study and treatment of analysed lipid measures. The long-term LDL-C and HDL-C profiles were analysed using a restricted maximum likelihood-based mixed model for repeated measures, which included treatment, visit and the treatment-by-visit interaction as fixed categorical effects, and baseline as fixed continuous effect to estimate change from baseline across postbaseline visits after modelling three covariance structures (the heterogeneous autoregressive, compound symmetry and the Toeplitz) and selecting the variance-covariance structure with the smallest Akaike information criterion. ANCOVA was also used to assess the NMR lipoprotein particle parameters and GlycA in RA-BEAM.

For patients who initiated statins during the study, lipid values were evaluated at baseline, initiation of statin therapy and the end of statin treatment or the analysis period. Analysis of variance containing explanatory terms for study and treatment was used. In addition, a subgroup analysis for change from baseline in total cholesterol, LDL-C, HDL-C and triglycerides based on subgroups defined by baseline statin use was performed using an ANCOVA model, with explanatory terms for baseline value, study, treatment, subgroup and treatment-by-subgroup interaction.

The Framingham Risk Score and Reynolds Risk Score were examined from each phase III study using ANCOVA, which included treatment and the baseline measurement as independent variables, and change from baseline as the dependent variable. The association between MACE and LDL-C change was examined using a waterfall plot. The waterfall plot was generated with each individual patient's change of LDL-C from baseline to maximum postbaseline measure, with data censored at treatment discontinuation, or statin therapy initiation or statin therapy change. Changes in LDL-C were ordered from right to left, representing the maximum decrease on the left end and maximum increase on the right end.

RESULTS

Baseline demographics and disease characteristics of patients from the six-study placebo-controlled set, RA-BEAM and the all-baricitinib RA data were generally comparable among treatment groups (table 1). Vital signs including blood pressure, pulse rate, weight and waist circumference were comparable among treatment groups (data not shown). In the all-baricitinib RA data, 37% of patients had a medical history of hypertension, 6% had diabetes at baseline and 31% were obese (≥30 kg/m^2). Previous studies of the phase III trials reported small increases in creatinine with baricitinib treatment.[2–5]

Lipid profile

In the six-study placebo-controlled set, baricitinib was associated with dose-dependent increases across all lipid

Table 1 Baseline demographics and disease characteristics in the six-study placebo-controlled set, RA-BEAM, and the all-baricitinib RA set

	Six-study placebo-controlled set			RA-BEAM			
	Placebo (n=1070)	Baricitinib 4 mg (n=997)	Baricitinib 2 mg (n=479)	Placebo (n=488)	Baricitinib 4 mg (n=487)	Adalimumab (n=330)	All-baricitinib RA (n=3492)*
Age, years	52.9 (11.9)	53.7 (12.0)	53.2 (12.0)	53.4 (11.8)	53.5 (12.2)	52.9 (12.3)	52.9 (12.2)
≥65 years of age, n (%)	173 (16)	199 (20)	82 (17)	82 (17)	103 (21)	56 (17)	612 (18)
BMI, kg/m^2	27.8 (7.1)	28.0 (6.8)	29.0 (7.4)	26.7 (6.3)	26.8 (5.8)	26.4 (5.4)	27.7 (6.7)
Tobacco use, yes, n (%)	198 (19)	196 (20)	84 (18)	103 (21)	109 (22)	74 (22)	663 (20)
Female, n (%)	862 (81)	794 (80)	386 (81)	382 (78)	375 (77)	251 (76)	2760 (79)
Duration of RA†, years	8.9 (8.4)	8.9 (8.6)	9.0 (8.1)	8.9 (8.0)	8.7 (8.6)	8.3 (7.9)	7.7 (8.2)
Region, n (%)							
USA/Canada	240 (22)	225 (23)	162 (34)	39 (8)	40 (8)	26 (8)	840 (24)
Central/South America and Mexico	203 (19)	197 (20)	54 (11)	141 (29)	143 (29)	96 (29)	701 (20)
Asia (excluding Japan)	84 (8)	83 (8)	38 (8)	48 (10)	48 (10)	33 (10)	226 (7)
Japan	156 (15)	132 (13)	36 (8)	93 (191)	93 (19)	63 (19)	514 (15)
European Union	263 (25)	246 (25)	125 (26)	116 (24)	114 (23)	78 (24)	783 (22)
Rest of the world	124 (12)	114 (11)	64 (13)	51 (11)	49 (10)	34 (10)	428 (12)
hsCRP (mg/L), median (first, third quartiles)	9.9 (4.8, 24.4)	10.7 (5.6, 25.4)	9.3 (4.9, 23.1)	12.7 (6.1, 26.8)	13.6 (6.6, 30.4)	14.4 (7.2, 29.8)	9.1 (4.1, 22.1)*
ESR, mm/hour	46.2 (24.9)	46.0 (25.1)	43.8 (22.3)	49.2 (26.1)	49.3 (25.8)	48.4 (25.6)	42.4 (25.4)*
DAS28-ESR	6.34 (0.99)	6.38 (0.96)	6.40 (0.99)	6.4 (1.0)	6.5 (0.9)	6.4 (1.0)	5.7 (1.5)*
DAS28-hsCRP	5.63 (0.95)	5.69 (0.94)	5.69 (0.96)	5.7 (1.0)	5.8 (0.9)	5.8 (0.9)	5.1 (1.5)*

Data are mean (SD) unless otherwise stated.
*The n for the all-baricitinib RA group for disease activity characteristics is 3439; it is smaller than the n for baseline demographics because efficacy baseline measures are only available for phase II/III studies; efficacy baseline measures were not available for a phase I RA study.
†Time from RA diagnosis.
BMI, body mass index; DAS28, Disease Activity Score in 28 joints; ESR, erythrocyte sedimentation rate; hsCRP, high-sensitivity C-reactive protein; RA, rheumatoid arthritis.

measures from baseline to week 12. Levels remained stable across groups from week 12 to 24. Per cent increases were similar from baseline to both weeks 12 and 24 (figure 1). There was no significant change in the LDL-C:HDL-C ratio from baseline at week 12 (placebo=−0.02, baricitinib 2 mg=−0.03, baricitinib 4 mg=−0.02) or week 24 (placebo=−0.03, baricitinib 2 mg=−0.04, baricitinib 4 mg=−0.01). After the initial increase from baseline to week 12 in patients receiving baricitinib 4 mg, LDL-C and HDL-C remained stable through week 104 (online supplementary figure S1).

Correlation analyses with disease activity measures in the baricitinib 2 mg/4 mg analysis set revealed weak correlations between lipid changes and disease activity (online supplementary table S2). In patients treated with baricitinib in the RA-BEAM study, increases in LDL-C and HDL-C were correlated with

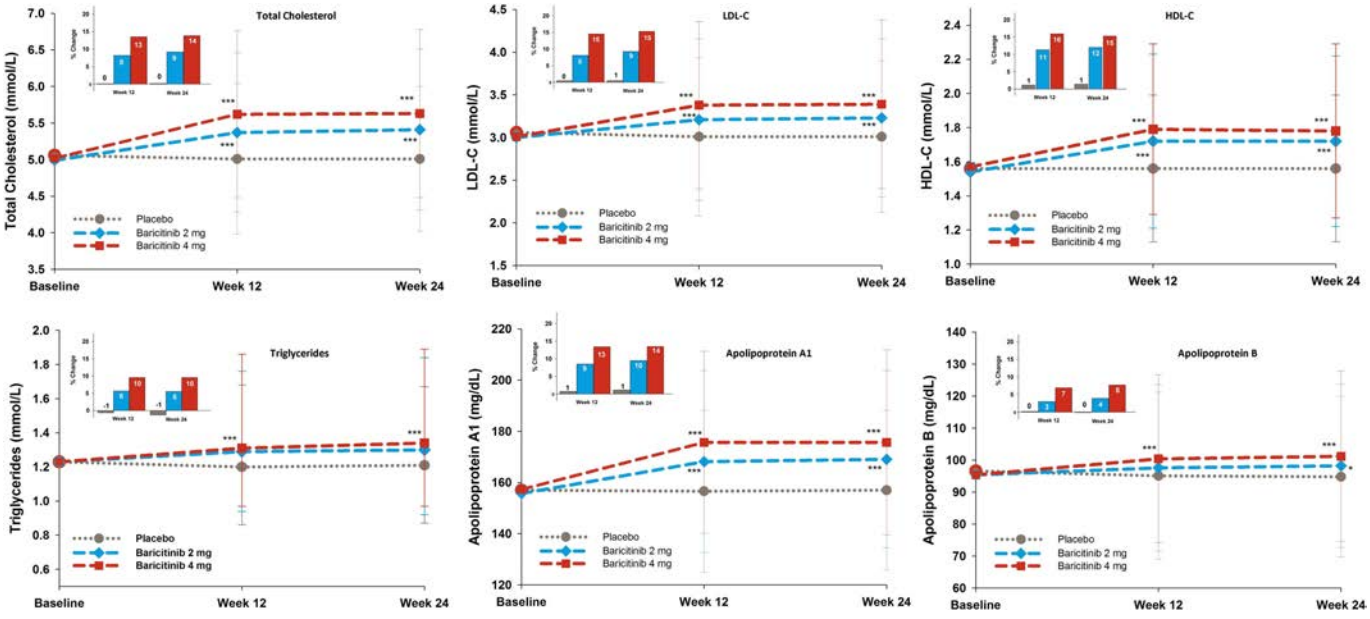

Figure 1 Lipid profile for total cholesterol, LDL-C, HDL-C, triglycerides, apolipoprotein A-I and apolipoprotein B in patients from the six-study placebo-controlled set. Data in line graphs are absolute values at baseline, week 12 and week 24 with data censored at rescue; mean (SD) for all lipids except triglycerides, which are median (25th, 75th percentiles). Patients on placebo who were rescued to baricitinib before week 24 were not included in this analysis. Inset bar charts show mean per cent change from baseline to weeks 12 and 24 with data censored at rescue. *P≤0.05, ***P≤0.001 versus placebo. HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

reductions in high-sensitivity C-reactive protein (hsCRP) and GlycA (online supplementary figure S2).

Lipid treatment-emergent highest values

Baseline abnormal LDL-C values (high or very high) were 11.8% in the placebo, 10.5% in the baricitinib 4 mg group and 8.7% in the baricitinib 2 mg group. Among patients in the baricitinib 4 mg group with a treatment-emergent increase in LDL-C, 92% of patients did not reach the maximum LDL-C category of ≥4.91 mmol/L, and 99% did not reach the maximum triglyceride category of ≥5.64 mmol/L (online supplementary table S3).

Changes in the NMR lipoprotein profile and GlycA

In RA-BEAM, there was a significant increase from baseline to week 12 in mean LDL-C in the baricitinib 4 mg group (0.42 mmol/L) and adalimumab group (0.20 mmol/L) compared with placebo (−0.06 mmol/L, P=0.001) and in mean HDL-C (baricitinib 4 mg, 0.24 mmol/L; adalimumab 0.10 mmol/L; placebo 0.00 mmol/L; P=0.001). Statistically significant increases in large LDL particle numbers and decreases in small LDL particle numbers were observed in baricitinib and adalimumab arms compared with placebo (table 2). There was a statistically significant increase in total LDL particle numbers in baricitinib compared with placebo. The increases in large LDL

Table 2 NMR lipoprotein profile: particle subfractions and GlycA in RA-BEAM

	Placebo n=488	Baricitinib 4 mg n=487	Adalimumab n=330
Mean number of particles (nmol/L)			
Total LDL			
Baseline	1178.60 (398.50)	1178.16 (335.46)	1165.15 (330.16)
Change from baseline at week 12	−21.31 (12.25)	15.77 (12.09)*++	−36.64 (14.77)
Large LDL			
Baseline	513.31 (216.87)	506.54 (225.29)	501.96 (227.72)
Change from baseline at week 12	−0.72 (8.20)	81.74 (8.09)***+++	39.49 (9.89)**
Small LDL			
Baseline	632.45 (415.25)	637.68 (374.39)	633.34 (372.48)
Change from baseline at week 12	−21.86 (15.09)	−80.26 (14.89)**	−82.86 (18.18)**
Medium small LDL			
Baseline	127.25 (83.21)	130.05 (77.97)	128.35 (77.36)
Change from baseline at week 12	−3.18 (3.15)	−16.29 (3.11)**	−15.47 (3.80)*
Very small LDL			
Baseline	505.26 (333.70)	507.64 (298.81)	504.94 (297.13)
Change from baseline at week 12	−18.76 (12.07)	−63.93 (11.91)**	−67.37 (14.55)*
Total HDL			
Baseline	28.88 (5.90)	28.69 (5.96)	28.48 (5.84)
Change from baseline at week 12	0.06 (0.22)	4.73 (0.21)***+++	1.93 (0.26)***
Large HDL			
Baseline	9.01 (3.41)	9.13 (3.48)	8.91 (3.56)
Change from baseline at week 12	0.00 (0.12)	0.98 (0.12)***++	0.41 (0.14)*
Medium HDL			
Baseline	2.82 (3.27)	2.68 (3.01)	2.89 (3.27)
Change from baseline at week 12	0.13 (0.14)	0.63 (0.14)*+++	−0.30 (0.17)*
Small HDL (nmol/L)			
Baseline	17.04 (5.59)	16.88 (5.66)	16.68 (5.54)
Change from baseline at week 12	−0.08 (0.21)	3.08 (0.21)***+++	1.86 (0.25)***
Mean particle size (nm)			
LDL particle size			
Baseline	21.34 (0.76)	21.29 (0.75)	21.28 (0.74)
Change from baseline at week 12	0.02 (0.03)	0.25 (0.03)***	0.23 (0.03)***
VLDL particle size			
Baseline	47.59 (9.29)	46.94 (9.09)	47.90 (11.61)
Change from baseline at week 12	0.57 (0.40)	2.34 (0.40)**	1.19 (0.49)
HDL particle size			
Baseline	9.34 (0.47)	9.33 (0.46)	9.36 (0.49)
Change from baseline at week 12	0.01 (0.01)	−0.02 (0.01)*	−0.00 (0.01)
Mean levels of GlycA (µmol/L)			
Baseline	508.9 (104.6)	517.2 (114.4)	513.9 (106.4)
Change from baseline at week 12	−13.6 (3.8)	−110.9 (3.8)***+	−98.7 (4.7)***

Baseline data are mean (SD); change from baseline data are least squares mean (SE).
*P≤0.05; **P≤0.01; ***P≤0.001 versus placebo. +P≤0.05; ++P≤0.01; +++P≤0.001 versus adalimumab.
HDL, high-density lipoprotein; LDL, low-density lipoprotein; NMR, nuclear magnetic resonance; RA, rheumatoid arthritis; VLDL, very-low-density lipoprotein.

Table 3 Change from baseline in lipids by baseline statin use in phase III studies up to 24 weeks with data censored at rescue

	Placebo		Baricitinib 2/4 mg		
	Non-statin (n=704)	Statin (n=84)	Non-statin (n=1054)	Statin (n=137)	Interaction P value
Total cholesterol, mmol/L					
Baseline	5.03 (1.00)	5.13 (1.20)	5.01 (1.01)	4.88 (1.05)	
Change from baseline to week 24	−0.01 (0.03)	0.17 (0.11)	0.59 (0.02)	0.63 (0.08)	0.505
LDL-C, mmol/L					
Baseline	3.04 (0.86)	3.00 (1.08)	3.03 (0.84)	2.81 (0.85)	
Change from baseline to week 24	−0.03 (0.02)	0.20 (0.10)	0.35 (0.02)	0.41 (0.07)	0.110
HDL-C, mmol/L					
Baseline	1.56 (0.42)	1.47 (0.40)	1.57 (0.42)	1.48 (0.36)	
Change from baseline to week 24	−0.00 (0.01)	0.00 (0.03)	0.20 (0.01)	0.24 (0.02)	0.454
Triglycerides, mmol/L					
Baseline	1.38 (0.71)	1.93 (1.83)	1.38 (0.74)	1.72 (0.96)	
Change from baseline to week 24	−0.01 (0.03)	−0.01 (0.12)	0.15 (0.02)	0.14 (0.09)	0.730

Baseline are mean (SD).
Change from baseline data are least squares mean (SE).
HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

particles and decreases in the smaller LDL particles produced an overall mean increase at 12 weeks in LDL size for baricitinib (0.25 nm) and adalimumab (0.23 nm) that was significantly greater than placebo (0.02 nm; P=0.001) (table 2).

Total HDL particle numbers increased with statistically significant elevations in large, medium and small HDL particles for baricitinib and in large and small HDL particles for adalimumab compared with placebo (table 2). The overall impact on mean HDL particle size for baricitinib was a modest decrease at week 12 (−0.02 nm), which was not observed for adalimumab (0.00 nm). A statistically significant within-group increase from baseline in the mean very-low-density lipoprotein particle size was observed in baricitinib (2.34 nmol/L) and adalimumab (1.19 nmol/L); the increase in baricitinib 4 mg was statistically significant compared with placebo (0.57 nmol/L; P=0.002) (table 2). There were dose-dependent decreases in GlycA levels from baseline to week 12 in JADA (−47.30, −49.80, −58.46 and −80.55 µmol/L in the baricitinib 1, 2, 4 and 8 mg dose groups, respectively). In RA-BEAM at week 12, GlycA levels in patients treated with baricitinib decreased significantly compared with placebo or adalimumab (table 2). There were similar reductions in GlycA with baricitinib regardless of baseline statin use (online supplementary table S4).

Statin use

Approximately 10% (n=221) of patients were on statin therapy at baseline. In the baricitinib 2/4 mg group, total cholesterol, LDL-C, HDL-C and triglycerides increased from baseline in both statin users and non-users (table 3). In the placebo group, total cholesterol, LDL-C, HDL-C and triglycerides remained essentially unchanged in non-statin users, while there was a modest increase from baseline in total cholesterol and LDL-C in statin users without much of a change in HDL-C or triglycerides (table 3). The interaction effect between study treatment and baseline statin subgroup was non-significant (P>0.1), suggesting that baseline statin use did not modify the effect of baricitinib on lipids.

Twenty patients in the placebo group, and 58 patients in the baricitinib 4 mg and 25 patients in the baricitinib 2 mg long-term cohorts initiated statin therapy after starting baricitinib and had lipid data available at baseline, statin initiation and the end of statin treatment. Figure 2 shows changes in lipid levels for these patients at each of these time points. The effects of statin therapy on LDL-C, total cholesterol, triglycerides and apolipoprotein B lipid levels were comparable in the baricitinib and placebo groups.

Cardiovascular risk scores and MACE

By baseline Framingham Risk Score, the majority of patients aged 30–74 years with no prior cardiovascular disease had low or intermediate risk in the phase III studies in each treatment group; less than 12% were considered high risk. There were no statistically significant within-group changes from baseline or statistically significant differences between groups (baricitinib 4 mg, placebo or adalimumab) (table 4). For the Reynolds Risk Score, most non-diabetic patients aged 45–80 with no prior cardiovascular disease (any hsCRP status and the subgroup with baseline hsCRP ≤20 mg/L) had baseline scores of low or low-to-moderate risk in the phase III studies. Less than 13% of patients were considered moderate to high or high risk. There was a statistically significant within-group decrease in Reynolds Risk Score for baricitinib groups in all three studies and for adalimumab in RA-BEAM as well as placebo in RA-BUILD. Only in RA-BEAM were the decreases significantly larger in the baricitinib group compared with placebo (table 4).

In the all-baricitinib RA-MACE set, 25 patients had positively adjudicated MACE (incidence rate=0.57 per 100 patient-years; patient-years of exposure=4402). There was no observed association between change in LDL-C and occurrence of MACE (online supplementary figure S3).

DISCUSSION

RA confers increased risk of cardiovascular events that are not explained fully by factors that contribute to cardiovascular risk in the general population. This has led to the suggestion that inflammation mediates direct effects on cardiovascular risk in RA and that interventions that modulate inflammatory burden may favourably influence the risk of a vascular event. Specifically, MTX is associated with reduced cardiovascular mortality in RA.[29] Tumour necrosis factor (TNF) inhibitors are similarly associated with reduced vascular mortality and event rates.[30]

Referred to as the RA lipid paradox, active disease is associated with 'lower than expected' cholesterol levels that in turn

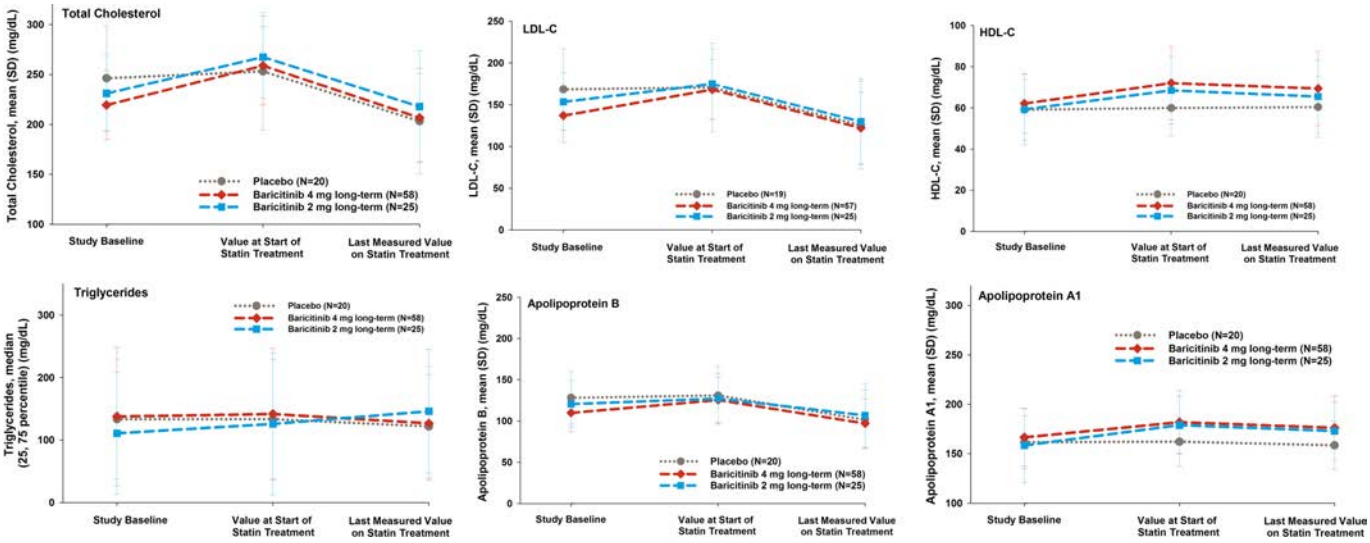

Figure 2 Lipid profile in patients who initiated statins during the phase III studies of the six-study placebo-controlled set including data in the long-term RA-BEYOND study for the baricitinib doses. Data are absolute values at three milestones: baseline, initiation of statin therapy and end of statin treatment or study period; mean (SD) for all lipids except triglycerides, which are median (25th, 75th percentiles). HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

may 'normalize' on effective management of the inflammatory disease state,[31 32] having several implications. First, it is difficult to determine the long-term impact of lipid changes in the context of treatment of active inflammation as in RA. For example, some effects of baricitinib, especially given the behaviour of the adalimumab control arm, likely do reflect the impact of normalisation of cholesterol levels. However, like tocilizumab and tofacitinib, the magnitude of change seems greater and more consistent for baricitinib than for TNF inhibitors and csDMARDs in historical comparator studies, and is likely to be target specific. However, in a recent clinical trial analysing MACE in patients with RA, no differences in event rates were observed when comparing tocilizumab with etanercept.[33] In our analysis, in general, we found low MACE rates with no increase over time and no apparent association between LDL-C change and MACE at the individual patient level. These data are not to be overinterpreted, but they do reassure that no immediate clinical effect is manifest on lipid changes in an at-risk population—about 12% of patients entering these studies were already in a high-risk category on recruitment. In this respect, long-term observational data will be important across the field and distinct mechanisms of action.

Overall trends in baricitinib NMR results observed previously were confirmed in this analysis of phase III data from RA-BEAM, with a few exceptions. In the phase II JADA study, there was a trend towards significance for decreases of small, medium small and very small LDL particles at 12 weeks[12]; in RA-BEAM these differences were all significant. Also, in JADA there was no increase in total LDL particle number; in RA-BEAM there was a small, but significant, increase in total LDL particle number in baricitinib versus placebo and adalimumab.

One possible explanation for the mechanism by which baricitinib and related interventions increase LDL particle size could be the increased activity of lipases such as phospholipase A2, hepatic lipase, lipoprotein lipase and endothelial lipase, which are reported to be increased in chronic inflammatory states.[34–37] Lipase levels were not investigated in this analysis, but it is possible that some lipases, specifically sPLA2, an acute phase reactant, could be reduced by an overall baricitinib-mediated reduction in systemic inflammation and thereby reduce the rate of lipoprotein remodelling. A reduction in inflammation-stimulated lipase activity could then contribute to a shift in the equilibrium towards larger lipoprotein particles. This

Table 4 Change in cardiovascular risk scores from baseline to week 24 with data censored at rescue

	RA-BEAM			RA-BUILD			RA-BEACON		
	Placebo (n=488)	Baricitinib 4 mg (n=487)	Adalimumab (n=330)	Placebo (n=228)	Baricitinib 2 mg (n=229)	Baricitinib 4 mg (n=227)	Placebo (n=126)	Baricitinib 2 mg (n=127)	Baricitinib 4 mg (n=137)
Framingham Risk Score									
Baseline	9.9 (9.9)	8.8 (9.6)	9.6 (10.0)	7.7 (6.8)	7.6 (6.4)	7.5 (6.6)	8.5 (6.3)	8.0 (6.4)	8.2 (8.0)
Change from baseline to week 24	0.02 (0.19)	0.28 (0.17)	−0.07 (0.21)	−0.30 (0.25)	0.02 (0.22)	−0.01 (0.22)	−0.24 (0.39)	−0.46 (0.34)	0.63 (0.32)
Reynolds Risk Score									
Baseline	5.4 (6.7)	4.5 (4.6)	5.3 (6.1)	4.7 (5.0)	3.9 (3.6)	4.4 (4.2)	5.1 (5.8)	4.3 (4.5)	4.6 (4.1)
Change from baseline to week 24	−0.11 (0.16)	−0.98 (0.15)*†	−1.08 (0.18)*†	−0.59 (0.20)‡	−0.66 (0.17)*	−0.47 (0.17)†	−0.32 (0.29)	−0.74 (0.26)‡	−0.69 (0.26)‡

Baseline are mean (SD).
Change from baseline data are least squares mean (SE).
*Significant within-group change from baseline (P<0.001).
†Significant change from baseline versus placebo (P<0.001).
‡Significant within-group change from baseline (P<0.01).
RA, rheumatoid arthritis.

shift in larger lipoprotein particles could reflect a reduction in the atherogenic potential of the overall LDL particles.

GlycA, a measure of glycosylated acute phase proteins, is an emerging inflammatory marker that may be useful for assessing disease activity and is associated with subclinical cardiovascular disease in patients with RA.[15 19] Baricitinib decreased GlycA dose dependently and reductions were seen regardless of baseline statin treatment. With recent studies suggesting GlycA as a marker for cardiovascular risk, these reductions in phase II and III studies in an RA population may portend a reduction in overall cardiovascular risk.

While in JADA there was no increase in LDL-C levels in patients on statins at baseline,[12] the current analysis of pooled baricitinib data showed that about 10% of patients already receiving statins on study entry demonstrated increases in lipids. It is not known if increasing statin dose or changing to a more potent statin would reverse this effect. Meanwhile, for patients starting a statin during the study, total cholesterol and LDL-C returned to baseline levels, whereas HDL-C remained elevated. Consistent with these findings, reduction in elevated cholesterol secondary to tofacitinib has previously been reported following initiation of atorvastatin.[38]

This study has some limitations. First, the observation period for the placebo-controlled comparisons is short (24 weeks) due to the need to limit the duration of placebo treatment. Therefore, long-term effects of baricitinib on lipid levels are based on within-group comparisons only. Second, the effects of baseline statin use on baricitinib-induced lipid changes did not account for postbaseline changes in statin dose or change to a different statin. Also, because a limited number of patients initiated statin therapy during the placebo-controlled period, we extended the analysis for statin effect to include data from the LTE for patients receiving baricitinib 2 and 4 mg treatment, while placebo data are based on 0–24 weeks; therefore, these effects are based on a non-randomised comparison of patients starting statin treatment. As a result, caution is warranted when interpreting the number of patients initiating statin as a proxy for the number of patients who need statin therapy. Third, while the majority of these analyses are from pooled data from multiple baricitinib studies, the NMR data were only collected and assessed in one phase III study in MTX-IR patients (RA-BEAM) and therefore we could not present these results from the broader patient population across the baricitinib programme. We used the Framingham Risk Score and Reynolds Risk Score to characterise the change of cardiovascular risk, which may not reflect well the cardiovascular risk in our target population given the lipid paradox in patients with RA. Additionally, the duration of follow-up for cardiovascular events in our analysis is limited to 24 weeks in the placebo-controlled portion of the studies and limited by the data cut-off date in the LTE. We continue to monitor cardiovascular events in extension studies.

In summary, increases in circulating lipid levels were seen following treatment with baricitinib that plateaued by 12 weeks of treatment. Increases in HDL occurred across all particle sizes whereas LDL increases were confined to larger particles, with small LDL particle levels decreasing. LDL increases reversed in response to statin therapy. These findings may reflect a positive impact on aspects of the lipid paradox observed in RA. However, further analyses of the effects of baricitinib on cholesterol and lipoprotein metabolism, as well as continued evaluation of cardiovascular event rates during long-term treatment, are warranted to further characterise these findings and their possible clinical implications.

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Patient consent Obtained.

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REFERENCES

1 Fridman JS, Scherle PA, Collins R, *et al*. Selective inhibition of JAK1 and JAK2 is efficacious in rodent models of arthritis: preclinical characterization of INCB028050. *J Immunol* 2010;184:5298–307.
2 Fleischmann R, Schiff M, van der Heijde D, *et al*. Baricitinib, Methotrexate, or Combination in Patients With Rheumatoid Arthritis and No or Limited Prior Disease-Modifying Antirheumatic Drug Treatment. *Arthritis Rheumatol* 2017;69:506–17.
3 Taylor PC, Keystone EC, van der Heijde D, *et al*. Baricitinib versus placebo or adalimumab in rheumatoid arthritis. *N Engl J Med* 2017;376:652–62.
4 Dougados M, van der Heijde D, Chen YC, *et al*. Baricitinib in patients with inadequate response or intolerance to conventional synthetic DMARDs: results from the RA-BUILD study. *Ann Rheum Dis* 2017;76:88–95.
5 Genovese MC, Kremer J, Zamani O, *et al*. Baricitinib in Patients with Refractory Rheumatoid Arthritis. *N Engl J Med* 2016;374:1243–52.
6 Robertson J, Peters MJ, McInnes IB, *et al*. Changes in lipid levels with inflammation and therapy in RA: a maturing paradigm. *Nat Rev Rheumatol* 2013;9:513–23.
7 O'Neill F, Charakida M, Topham E, *et al*. Anti-inflammatory treatment improves high-density lipoprotein function in rheumatoid arthritis. *Heart* 2017;103:766–73.
8 Tam LS, Tomlinson B, Chu TT, *et al*. Impact of TNF inhibition on insulin resistance and lipids levels in patients with rheumatoid arthritis. *Clin Rheumatol* 2007;26:1495–8.
9 Navarro-Millán I, Charles-Schoeman C, Yang S, *et al*. Changes in lipoproteins associated with methotrexate or combination therapy in early rheumatoid arthritis: results from the treatment of early rheumatoid arthritis trial. *Arthritis Rheum* 2013;65:1430–8.
10 Genovese MC, McKay JD, Nasonov EL, *et al*. Interleukin-6 receptor inhibition with tocilizumab reduces disease activity in rheumatoid arthritis with inadequate response to disease-modifying antirheumatic drugs: the tocilizumab in combination with traditional disease-modifying antirheumatic drug therapy study. *Arthritis Rheum* 2008;58:2968–80.
11 Charles-Schoeman C, Fleischmann R, Davignon J, *et al*. Potential mechanisms leading to the abnormal lipid profile in patients with rheumatoid arthritis versus healthy volunteers and reversal by tofacitinib. *Arthritis Rheumatol* 2015;67:616–25.
12 Kremer JM, Genovese MC, Keystone E, *et al*. Effects of Baricitinib on Lipid, Apolipoprotein, and Lipoprotein Particle Profiles in a Phase IIb Study of Patients With Active Rheumatoid Arthritis. *Arthritis Rheumatol* 2017;69:943–52.
13 Rizzo M, Berneis K. Low-density lipoprotein size and cardiovascular risk assessment. *QJM* 2006;99:1–14.
14 Otvos JD, Shalaurova I, Wolak-Dinsmore J, *et al*. GlycA: a composite nuclear magnetic resonance biomarker of systemic inflammation. *Clin Chem* 2015;61:714–23.
15 Ormseth MJ, Chung CP, Oeser AM, *et al*. Utility of a novel inflammatory marker, GlycA, for assessment of rheumatoid arthritis disease activity and coronary atherosclerosis. *Arthritis Res Ther* 2015;17:117.
16 Bartlett DB, Connelly MA, AbouAssi H, *et al*. A novel inflammatory biomarker, GlycA, associates with disease activity in rheumatoid arthritis and cardio-metabolic risk in BMI-matched controls. *Arthritis Res Ther* 2016;18:86.
17 Connelly MA, Gruppen EG, Otvos JD, *et al*. Inflammatory glycoproteins in cardiometabolic disorders, autoimmune diseases and cancer. *Clin Chim Acta* 2016;459:177–86.
18 AbouAssi H, Connelly MA, Bateman LA, *et al*. Does a lack of physical activity explain the rheumatoid arthritis lipid profile? *Lipids Health Dis* 2017;16:39.
19 Joshi AA, Lerman JB, Aberra TM, *et al*. GlycA is a novel biomarker of inflammation and subclinical cardiovascular disease in Psoriasis. *Circ Res* 2016;119:1242–53.
20 Greenwald MW, Fidelus-Gort R, Levy R, *et al*. A randomized dose-ranging, placebo-controlled study of INCB028050, a selective JAK1 and JAK2 inhibitor in subjects with active rheumatoid arthritis. *Arthritis Rheum* 2010;62(Suppl 10):2172.
21 Keystone EC, Taylor PC, Drescher E, *et al*. Safety and efficacy of baricitinib at 24 weeks in patients with rheumatoid arthritis who have had an inadequate response to methotrexate. *Ann Rheum Dis* 2015;74:333–40.
22 Tanaka Y, Emoto K, Cai Z, *et al*. Efficacy and Safety of Baricitinib in Japanese Patients with Active Rheumatoid Arthritis Receiving Background Methotrexate Therapy: A 12-week, Double-blind, Randomized Placebo-controlled Study. *J Rheumatol* 2016;43:504–11.
23 Smolen J, Genovese M, Takeuchi T, *et al*. THU0166 Safety Profile of Baricitinib in Patients with Active RA: An Integrated Analysis: Table 1. *Ann Rheum Dis* 2016;75(Suppl 2):243.2–4.
24 Jacobson TA, Ito MK, Maki KC, *et al*. National Lipid Association recommendations for patient-centered management of dyslipidemia: part 1 - executive summary. *J Clin Lipidol* 2014;8:473–88.
25 Jeyarajah EJ, Cromwell WC, Otvos JD. Lipoprotein particle analysis by nuclear magnetic resonance spectroscopy. *Clin Lab Med* 2006;26:847–70.
26 Wilson PW, D'Agostino RB, Levy D, *et al*. Prediction of coronary heart disease using risk factor categories. *Circulation* 1998;97:1837–47.
27 Ridker PM, Buring JE, Rifai N, *et al*. Development and validation of improved algorithms for the assessment of global cardiovascular risk in women: the Reynolds Risk Score. *JAMA* 2007;297:611–9.
28 Ridker PM, Paynter NP, Rifai N, *et al*. C-reactive protein and parental history improve global cardiovascular risk prediction: the Reynolds Risk Score for men. *Circulation* 2008;118:2243–51.
29 Marks JL, Edwards CJ. Protective effect of methotrexate in patients with rheumatoid arthritis and cardiovascular comorbidity. *Ther Adv Musculoskelet Dis* 2012;4:149–57.
30 Greenberg JD, Kremer JM, Curtis JR, *et al*. CORRONA Investigators. Tumour necrosis factor antagonist use and associated risk reduction of cardiovascular events among patients with rheumatoid arthritis. *Ann Rheum Dis* 2011;70:576–82.
31 Myasoedova E, Crowson CS, Kremers HM, *et al*. Lipid paradox in rheumatoid arthritis: the impact of serum lipid measures and systemic inflammation on the risk of cardiovascular disease. *Ann Rheum Dis* 2011;70:482–7.
32 Choy E, Ganeshalingam K, Semb AG, *et al*. Cardiovascular risk in rheumatoid arthritis: recent advances in the understanding of the pivotal role of inflammation, risk predictors and the impact of treatment. *Rheumatology* 2014;53:2143–54.
33 Giles JT, Sattar N, Gabriel SE, *et al*. Cardiovascular risk in rheumatoid arthritis: recent advances in the understanding of the pivotal role of inflammation, risk predictors and the impact of treatment. *Arthritis Rheumatol* 2016;68(Suppl 10):4357–9.
34 McInnes IB, Kim HY, Lee SH, *et al*. Open-label tofacitinib and double-blind atorvastatin in rheumatoid arthritis patients: a randomised study. *Ann Rheum Dis* 2014;73:124–31.
35 Kitas GD, Gabriel SE. Cardiovascular disease in rheumatoid arthritis: state of the art and future perspectives. *Ann Rheum Dis* 2011;70:8–14.
36 Annema W, Tietge UJ. Role of hepatic lipase and endothelial lipase in high-density lipoprotein-mediated reverse cholesterol transport. *Curr Atheroscler Rep* 2011;13:257–65.
37 McInnes IB, Thompson L, Giles JT, *et al*. Effect of interleukin-6 receptor blockade on surrogates of vascular risk in rheumatoid arthritis: MEASURE, a randomised, placebo-controlled study. *Ann Rheum Dis* 2015;74:694–702.
38 Yue X, Wu M, Jiang H, *et al*. Endothelial lipase is upregulated by interleukin-6 partly via the p38 MAPK and p65 NF-κB signaling pathways. *Mol Med Rep* 2016;14:1979–85.

EXTENDED REPORT

Time spent in inactive disease before MTX withdrawal is relevant with regard to the flare risk in patients with JIA

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ABSTRACT

Objectives To determine the reasons of methotrexate (MTX) discontinuation, frequency of adverse events (AE) and whether the time in inactive disease before MTX withdrawal disease is associated with the risk of disease flare.

Methods Patients with juvenile idiopathic arthritis (JIA) beginning treatment with MTX were prospectively observed in the national JIA biologic register Biologika in der Kinderrheumatologie/Biologics in Paediatric Rheumatology and its follow-up register Juvenile arthritis Methotrexate/Biologics long-term Observation. Inactive disease was defined by a clinical Juvenile Arthritis Disease Activity Score ≤1, flare after MTX discontinuation by reoccurrence of at least moderate disease activity or restart of treatment with a disease-modifying antirheumatic drug .

Results MTX treatment was initiated in 1514 patients after a mean disease duration of 2.1 years (SD=2.8). 40% of the patients experienced oligoarticular onset of JIA. MTX was discontinued in 982 (64.9%) patients. Ineffectiveness (36.9%) and achieving inactive disease (32.1%) were the most common reasons. Among the latter (n=316), 184 (58.2%) patients experienced a flare on follow-up. The likelihood of a flare was a function of time in inactive disease prior to MTX discontinuation (HR 0.95; 95% CI 0.92 to 0.97). Patients with inactive disease for longer than 12 months had a significantly lower flare rate (58 of 119, 48.7%; HR 0.48; 95% CI 0.34 to 0.69). The most frequently reported AE was MTX intolerance, including nausea, aversion and vomiting, accounting for 441 events (13.0 events/100 exposure years) in 307 (20.3%) patients.

Conclusions Patients who spent at least 12 months in inactive disease before MTX discontinuation had a significantly lower flare rate.

Juvenile idiopathic arthritis (JIA) is the most common chronic inflammatory rheumatological disease in childhood. JIA comprises a group of heterogeneous forms of arthritis characterised by joint inflammation persisting longer than 6 weeks and beginning before the age of 16 with unknown aetiology. According to the classification criteria of the International League of Associations for Rheumatology (ILAR),[1] the term JIA includes seven mutually exclusive categories with differences in clinical presentation, disease course and treatment response.

Methotrexate (MTX) has been the most widely used first-line disease-modifying antirheumatic drug (DMARD) in the treatment of JIA for more than 25 years. The efficacy of MTX in JIA was demonstrated in randomised controlled trials[2 3] and cohort studies.[4 5] Serious adverse events (SAEs) rarely occur during MTX treatment. However, high adverse event (AE) rates have been reported for MTX, including gastrointestinal AEs, such as abdominal discomfort and vomiting.[3 6 7] Gastrointestinal AEs and the conditioned response to MTX[8] are included in the broader concept of subjective MTX intolerance. MTX intolerance increases with time of MTX therapy, negatively influences the quality of life of patients[9] and may lead to treatment discontinuation and non-compliance.[10 11] MTX intolerance may impact on the treatment goal of inducing early disease remission in JIA by treatment discontinuation and non-compliance. Therefore, it is important to develop strategies for discontinuing MTX that minimise the risk of disease flare after attaining clinical inactive disease (CID).

The objective of this study was to investigate predictors of developing active disease after discontinuation of MTX due to CID, as well as the onset of AEs under MTX in two large prospective German JIA registers: Biologika in der Kinderrheumatologie/Biologics in Paediatric Rheumatology (BiKeR), and its follow-up register, Juvenile arthritis Methotrexate/Biologics long-term Observation (JuMBO).

PATIENTS AND METHODS

The paediatric registry BiKeR[12] and its follow-up registry JuMBO[13] are ongoing, prospective observational cohort studies. Both studies collect data on the safety, effectiveness and treatment adherence for biologic (b) and conventional (cs) DMARDs in patients with definite JIA according to ILAR.[14] In BiKeR, a cohort of biologic-naive patients starting treatment with MTX were recruited from 2005 to 2011.[4 5] Young adults were transferred from BiKeR to the follow-up registry JuMBO when they left paediatric care or were 18 years of age. More detailed information about the study design of JuMBO can be found elsewhere.[13] Only patients who were enrolled in BiKeR at the start of MTX (MTX control cohort) were included in our study (figure 1). All patients provided informed consent.

Patients were prospectively assessed semi-annually by physician-reported and patient-reported outcomes via questionnaires. The physicians reported about details on current DMARD use (start and discontinuation dates) and provided information about the reasons for its discontinuation

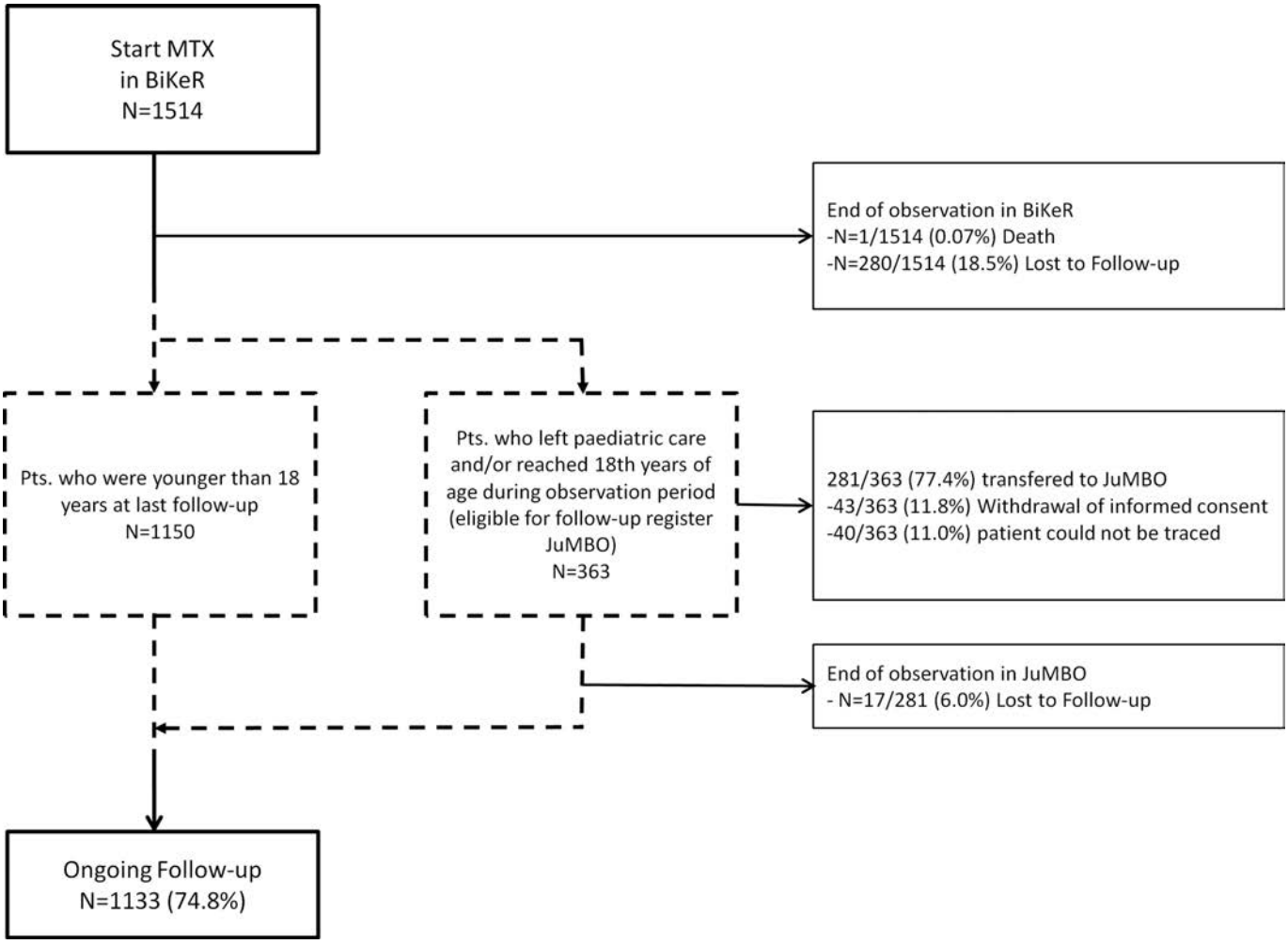

Figure 1 Patient flowchart. BiKeR, Biologika in der Kinderrheumatologie/Biologics in Paediatric Rheumatology; JuMBO, Juvenile arthritis Methotrexate/Biologics long-term Observation; MTX, methotrexate.

during follow-up. Physicians examined the health status of the patient, including the current disease activity (global assessment), 72-joint count, erythrocyte sedimentation rate and AEs during treatment. The Medical Dictionary MedDRA V.18.1 was used to code all events. We counted the AEs nausea, aversion, vomiting and drug intolerance under the term MTX intolerance as a proxy measure. SAEs were defined as events that were fatal or life threatening led to a persistent or significant disability or incapacity or required a prolonged hospitalisation. Parents were requested to assess the activity of their child's disease (global assessment) and current pain level using a 10-point visual analogue scale. The clinical Juvenile Arthritis Disease Activity Score (cJADAS10) was calculated.[15]

CID was defined by a cJADAS10 ≤ 1.[15] The time in CID before MTX discontinuation started with the visit with the first documentation of a cJADAS10 less than or equal to one. Flare was defined as reoccurrence of at least moderate disease activity (cJADAS10 >1.5 or 2.5 for oligoarticular or polyarticular disease)[15] or restarting a DMARD. The date of flare is either noted as the first physician visit when the patient first exhibited at least moderate disease activity or the date of restart of csDMARD and/or bDMARD. The cJADAS10 was missing in 12.8% of visits across all patients and visits. The details about handling of missing cJADAS10 are described in the online Supplementary material 1.

STATISTICS

Univariable and multivariable Cox proportional hazard models were used to investigate potential predictor variables for the two outcome variables of MTX discontinuation after attaining CID and the risk of a disease flare in follow-up. The known correlates JIA category, disease duration, sex, cJADAS10 at MTX start and antinuclear antibodies positivity were included in the analyses as predictor variables. The proportional hazards assumption was tested by Schoenfeld residuals. A sensitivity analysis included the analysis of the risk for flare by the multiple imputation technique for missing data as described in the online Supplementary material 1. To estimate the risk for AEs under MTX treatment, event rates per 100 MTX exposure years (EY) were estimated with their exact Poisson CIs. The level of significance was set at 5%. Statistical analyses were conducted by SAS V.9.4.

RESULTS

Study population

A total of 1514 patients with JIA (67.5% female) were included in the MTX register with a mean disease duration of 2.1 years (SD=2.8) and a mean age at MTX start of 9.8 years (SD=4.8; table 1). The mean disease duration at MTX initiation was 2.7 years (SD=3.2) for patients with oligoarthritis and 1.8 (SD=2.4) years for the other JIA categories. More than half of the sample (53.8%) received oral application of MTX at

Table 1 Sociodemographic and clinical characteristics of the study population at methotrexate treatment start

	N_{total}	Total n = 1514
Age, years, mean(SD)	1514	9.8 (4.8)
Female gender, N (%)	1514	1022 (67.5 %)
JIA category, N (%)	1514	
Systemic JIA		57 (3.8 %)
RF-negative polyarthritis		415 (27.4 %)
RF-positive polyarthritis		52 (3.4 %)
Persistent oligoarthritis		404 (26.7 %)
Extended oligoarthritis		196 (13.0 %)
Enthesitis-related arthritis		207 (13.7 %)
Psoriatic arthritis		137 (9.1 %)
Other arthritis		46 (3.0 %)
Disease duration, years, mean (SD)/median (IQR)	1508	2.1 (2.8)/0.9 (0.4–2.8)
Age at disease onset, years, mean(SD)	1508	7.7 (4.6)
Current uveitis, N (%)*	255	11 (4.9%)
cJADAS10, mean(SD)/median (IQR)	1251	14.0 (9.8)/12.4 (8.2–17.2)
Physician assessed disease activity, VAS score, mean(SD)	1405	47.3 (25.8)
Number of active joints, mean(SD)/median (IQR)	1427	5.8 (7.5)/3 (2–7)
CRP, mg/dL, mean(SD)/median (IQR)	1338	13.9 (28.4)/4.0 (1.1–13.0)
ESR, mm/1 hour, mean(SD)/median (IQR)	1401	24.1 (22.9)/16 (8.0–32.0)
Positive ANA test at baseline, N (%)	1460	729 (49.9 %)
Positive HLA-B27 test, N (%)	1297	264 (20.4 %)
NSAID, N (%)	1514	1291 (85.3%)
Systemic glucocorticoids, N (%)	1514	285 (18.8%)
Intra-articular glucocorticoids, N (%)	1514	464 (30.7%)
Previous DMARD treatment, N (%)	1514	
Sulfasalazine		66 (4.4 %)
Chloroquine/hydroxychloroquine		52 (3.4 %)
Azathioprine		14 (0.9 %)
Ciclosporin A		4 (0.3 %)
Leflunomide		4 (0.3 %)
Mycophenolate mofetil		1 (0.1 %)
Number of previous DMARDS used, mean(SD)	1514	0.09 (0.31)
Patient assessed disease activity, VAS score, mean(SD)	1263	45.6 (27.3)
C-HAQ total score, mean(SD)/median (IQR)	1362	0.58 (0.60)/0.38 (0.13–0.88)
Patient reported pain, VAS score, mean(SD)	1276	36.9 (27.9)
Patient-reported well-being, VAS score, mean(SD)	1268	35.9 (26.0)

*Documentation of current uveitis was available since 2009.
ANA, antinuclear antibody; C-HAQ, Childhood Health Assessment Questionnaire; cJADAS10, clinical Juvenile Arthritis Disease Activity Score; CRP, C-reactive protein; DMARD, disease-modifying antirheumatic drug; ESR, erythrocyte sedimentation rate; HLA, human leucocyte antigen; JIA, juvenile idiopathic arthritis; N_{total}, number of patients with valid information; NSAID, non-steroidal anti-inflammatory drugs; RF, rheumatoid factor; VAS, visual analogue scale.

treatment start, and the median dosage was 11.4 (SD=2.6) mg/m^2. In addition, 85.3% patients were treated with non-steroidal anti-inflammatory drugs (NSAIDs), 18.8% were treated with oral glucocorticoids and 30.7% with intra-articular glucocorticoids before study inclusion. The total mean observation time was 3.6 years (SD=2.1, min=3 months, max=10.2) in our cohort.

Risk of disease flare among patients who discontinued MTX due to CID

MTX therapy was discontinued in 982 (64.9%) patients after a median treatment duration of 2.3 years (IQR 1.1–3.3) as reported in figure 2. Three hundred and twenty patients (21.2%) switched to a combination therapy of MTX and a bDMARD after a mean of 1.5 years (SD=1.4) during the first MTX treatment cycle due to ongoing active disease (mean cJADAS10 at bDMARD start of 10.7 (SD=8.4)). Ineffectiveness was the leading cause of discontinuation (figure 2). MTX monotherapy was restarted in 209 (21.3%) patients, 12 of 29 patients (41.4%, patients with a report about uveitis activity) had an active uveitis at therapy start while five patients (41.4% of 12) had a cJADAS10 ≤1. We identified 309 patients in our register who did not restart with either a csDMARD or a bDMARD in follow-up after MTX withdrawal.

Sixty-eight per cent of the patients (n=1030) ever experienced any period of CID on MTX. MTX was discontinued due to attaining CID in 316 patients (20.8%) after a mean treatment duration of 2.3 years (SD=1.1). Forty-five patients (14.2% of 316) of those had a mean cJADAS10 between 1.1 and 3 mainly caused by a patient's global above 1 (n=33 of 45, 73%) at MTX withdrawal. Disease duration before the initiation of MTX (HR 0.93; 95% CI 0.88 to 0.99) and cJADAS10 at MTX start (HR 0.98; 95% CI 0.96 to 0.99) were negatively associated with the likelihood of discontinuing MTX due to CID (table 2) in multivariable analysis. The disease course in patients who discontinued MTX due to CID was further investigated with a mean follow-up of 2.5 years (SD=1.6).

A total of 184 (58.2% of 316) patients experienced a flare after MTX discontinuation after a mean period of CID of 7.4 months (SD=9.7). The majority of those (n=115, 62.5%) required the initiation of treatment with a csDMARD or bDMARD (MTX monotherapy: n=104, 56.6%; etanercept monotherapy: n=8, 4.3%; others: n=3, 1.6%). Among those, 59 (56.7% of 115) patients were in a state of inactive or minimally active disease, while active uveitis was reported in six (85.7%) of seven patients with available information about uveitis at this time point. Approximately, three out of four patients (n=144, 78.3%) experienced a disease flare within the first 12 months after MTX discontinuation. The highest flare rate involved children with extended oligoarthritis (n=33 of 44, 75.0%; P=0.024) and rheumatoid factor (RF)-positive polyarthritis (n=5 of 7, 71.4%; P=0.359). Patients with persistent oligoarthritis exhibited a significantly increased flare rate (n=88 of 132, 66.7%; HR 1.58; 95% CI 1.18 to 2.11) in univariable analyses. We could not find a relevant difference in the mean time spent in CID before MTX withdrawal between patients with oral (mean=9.2 months, SD=7.7) and subcutaneous MTX (mean=8.6 months, SD=7.7). Patients with oral MTX showed a slightly higher flare rate (66.7%) compared with subcutaneous MTX (50.0%); however, this difference was not statistically significant. MTX was tapered starting from the initial dose to the last dose before withdrawal in 38% patients. Half of the patients experienced a dose reduction of at least 50%. The presence of antinuclear antibodies was positively associated with disease flare (HR 1.40; 95% CI 1.04 to 1.89) in univariate analyses. The likelihood of a disease flare in follow-up was negatively associated with time in CID under MTX treatment before its discontinuation (in months, HR 0.95; 95% CI 0.93 to 0.97; table 3). The flare rate was highest for patients with CID for less than 6 months prior to MTX withdrawal (84 of 117, 71.8%). Patients with a CID for longer than 12 months (mean 16.5 months, SD=3.9) prior

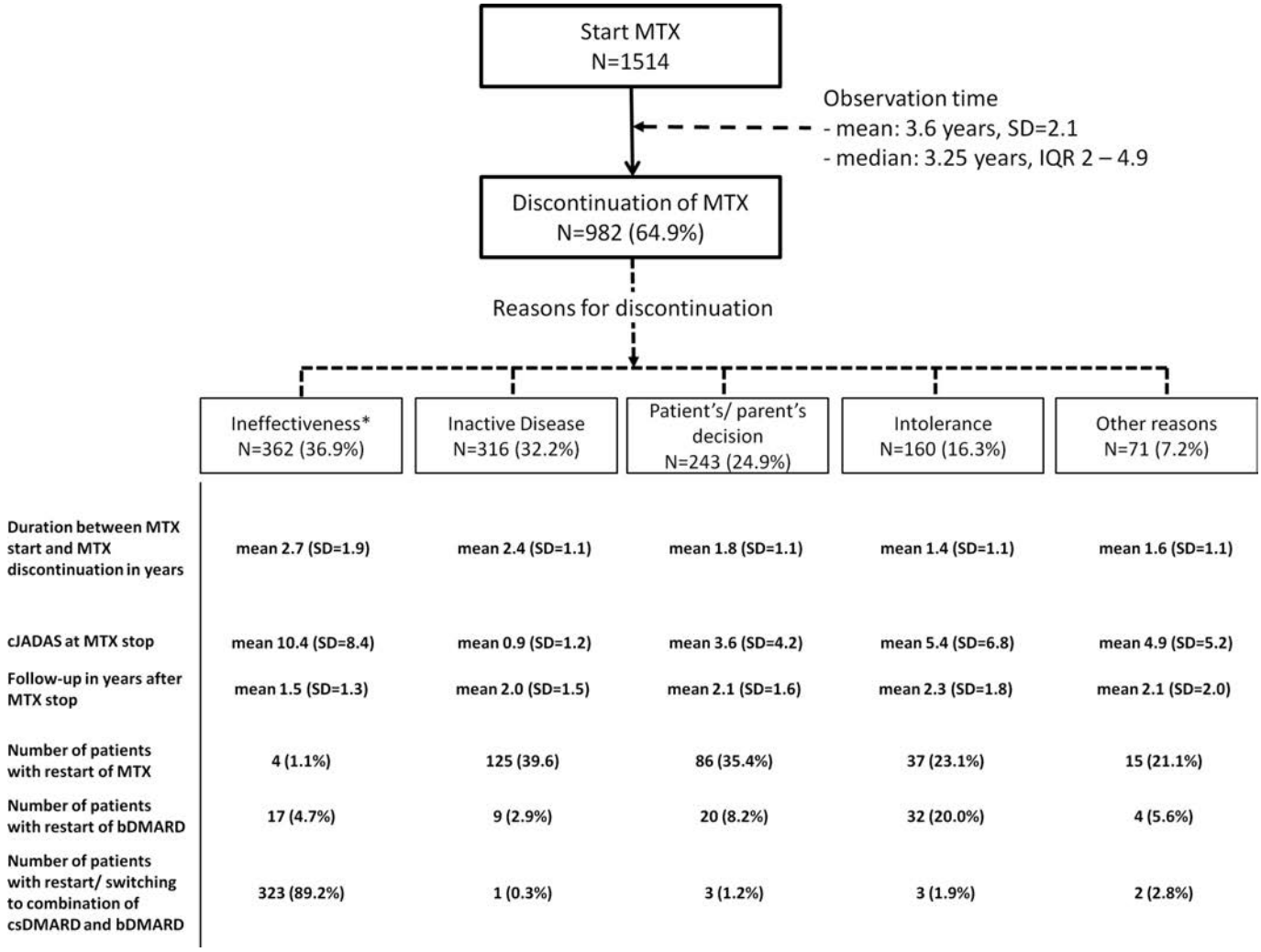

Figure 2 Reasons for MTX discontinuation (multiple reasons may be reported by the physician, *ineffectiveness refers to MTX discontinuation by the physician due to ineffectiveness or the adding of a biologic due to ineffectiveness). DMARD, disease-modifying antirheumatic drug; MTX, methotrexate.

Table 2 Multivariable predictors of MTX discontinuation once the patients achieved inactive diseases

	Univariable analyses			Multivariable analysis*		
	HR	95% CI	P value	HR	95% CI	P value
Female sex	0.94	0.74 to 1.19	0.613	1.00	0.77 to 1.30	0.995
Disease duration at MTX start, in years	0.92	0.88 to 0.97	0.002	0.93	0.88 to 0.99	0.014
Age at JIA onset, in years	1.00	0.98 to 1.03	0.750	1.00	0.98 to 1.03	0.786
ANA positivity	1.02	0.82 to 1.28	0.859	0.96	0.74 to 1.25	0.785
Systemic JIA	1.79	1.17 to 2.75	0.008	1.65	1.01 to 2.70	0.046
RF-negative polyarthritis	1.08	0.85 to 1.38	0.542	1.19	0.90 to 1.58	0.227
RF-positive polyarthritis	0.80	0.42 to 1.53	0.498	0.89	0.46 to 1.74	0.742
Persistent oligoarthritis	1.01	0.79 to 1.30	0.921	1.07	0.81 to 1.40	0.649
Extended oligoarthritis	0.72	0.53 to 0.98	0.035	0.79	0.56 to 1.10	0.164
Enthesitis-related arthritis	0.76	0.51 to 1.15	0.192	0.70	0.44 to 1.12	0.139
Psoriatic arthritis	1.03	0.72 to 1.48	0.876	0.99	0.66 to 1.47	0.946
cJADAS10 at MTX start	0.99	0.97 to 0.99	0.039	0.98	0.96 to 0.99	0.009
C-HAQ at MTX start	0.84	0.69 to 1.03	0.086	0.97	0.76 to 1.22	0.767

*All variables were entered into the multivariable analysis.
ANA, antinuclear antibody; C-HAQ, Childhood Health Assessment Questionnaire; cJADAS10, clinical Juvenile Arthritis Disease Activity Score; JIA, juvenile idiopathic arthritis; MTX, methotrexate; RF, rheumatoid factor.

Table 3 Multivariable predictors of disease flare in follow-up after MTX discontinuation once the patients achieved inactive diseases

	Model including time in inactive disease as continuous variable			Model including time in inactive disease as categorical variable		
	HR	95% CI	P value	HR	95% CI	P value
Time in inactive disease before MTX discontinuation						
In months	0.95	0.93 to 0.97	<0.0001	–	–	–
<6 months				1.00	–	–
Six to 12 months	–	–	–	0.61	0.41 to 0.91	0.016
More than 12 months	–	–	–	0.50	0.34 to 0.74	0.001
Female sex	0.76	0.53 to 1.08	0.125	0.75	0.53 to 1.07	0.114
Disease duration at MTX start, in years	1.04	0.97 to 1.11	0.290	1.03	0.97 to 1.10	0.326
ANA positivity	1.21	0.87 to 1.69	0.259	1.17	0.84 to 1.63	0.346
Systemic JIA	0.39	0.17 to 0.88	0.023	0.39	0.17 to 0.89	0.026
RF-negative polyarthritis	0.83	0.58 to 1.18	0.295	0.87	0.61 to 1.25	0.464
RF-positive polyarthritis	2.41	1.09 to 5.35	0.031	2.14	0.96 to 4.77	0.062
Persistent oligoarthritis	1.25	0.87 to 1.78	0.228	1.25	0.88 to 1.79	0.218
Extended oligoarthritis	1.14	0.75 to 1.72	0.547	1.19	0.79 to 1.80	0.406
Enthesitis-related arthritis	0.67	0.37 to 1.24	0.206	0.69	0.38 to 1.28	0.245
Psoriatic arthritis	0.63	0.36 to 1.13	0.122	0.65	0.37 to 1.17	0.150
cJADAS10 at MTX start	0.99	0.97 to 1.01	0.305	0.99	0.97 to 1.01	0.362
Duration of MTX treatment before withdrawal, in months	0.99	0.98 to 1.01	0.247	0.99	0.98 to 1.00	0.148

ANA, antinuclear antibody; cJADAS10, clinical Juvenile Arthritis Disease Activity Score; JIA, juvenile idiopathic arthritis; MTX, methotrexate; RF, rheumatoid factor.

to MTX discontinuation exhibited a significantly reduced flare rate (58 of 119, 48.7%; HR 0.50; 95% CI 0.34 to 0.74; table 3).

A sensitivity analysis was performed in patients with a documented disease flare or with the latest available follow-up in BiKeR (n=227). Among patients who did not meet the flare definition (n=91), six (6.5%) were treated with any NSAID or glucocorticoid after MTX withdrawal in follow-up (NSAID: 1, 1.0%; systemic glucocorticoids: 1, 1.0%; local glucocorticoid injections: 1, 1.0%). The estimated overall flare rate will be 190 (60.1%), when counting these six patients as flare. A second sensitivity analyses was conducted by using the multiple imputation technique to estimate the missing data in the analyses of the flare risk. This analysis yielded comparable results as reported above. We refer to the online Supplementary material 1 for details.

Table 4 Incidence of adverse events in patients with JIA treated with MTX

	n	Rate per 100 EY (95% CI)
Mortality	1	0.03 (0.01 to 0.11)
Acute lymphoblastic leukaemia	2	0.06 (0.01 to 0.16)
Adverse events	1.058	31.08 (29.24 to 32.98)
Serious adverse events	63	1.85 (1.42 to 2.33)
All infections	183	5.38 (4.60 to 6.15)
Medically important infections	13	0.38 (0.20 to 0.62)
Gastrointestinal toxicity	441	12.95 (11.75 to 14.16)
Nausea	271	7.96 (7.01 to 8.90)
Vomiting	81	2.38 (1.86 to 2.90)
Aversion	16	0.47 (0.24 to 0.70)
Increased transaminases	141	4.14 (3.46 to 4.83)
Headache	22	0.65 (0.41 to 0.94)
Uveitis	20	0.59 (0.36 to 0.87)
Leucopenia, neutropaenia	10	0.29 (0.14 to 0.50)
Alopecia	9	0.26 (0.12 to 0.46)
Fatigue	8	0.24 (0.10 to 0.42)
Exanthem	6	0.18 (0.06 to 0.34)

EY, exposure years.

AEs during treatment with MTX

AEs during the first treatment course with MTX were analysed with a mean treatment duration of 2 years (SD=1.5) accounting for 3.404 EY (table 4, see table S1 in the online Supplementary material 1). Concomitant treatment included NSAIDs (1339 EY), systemic glucocorticoids (440 EY) and local glucocorticoid injections. In total, 1.058 AEs (31.1 events/100 EY) were reported in 540 (35.7%) patients, among those were 63 SAE (1.9 events/100 EY). The most frequently reported AE was MTX intolerance, accounting for 441 events (13.0 events/100 EY) in 307 (20.3%) patients. There was no statistically significant difference between oral or subcutaneous MTX. MTX was discontinued in 34.3% patients with MTX intolerance. Infections were the second most reported AE (181, 5.3 events/100 EY) with 13 serious infections were recorded (0.4 events/100 EY). A total of two acute lymphocytic leukaemia (ALL; 0.06 events/100 EY) events were observed during follow-up. More details on both cases can be found in the online supplementary material 1.

DISCUSSION

MTX is the most widely used DMARD in the treatment of JIA and is very effective in many patients with JIA.[2 3 5 16] MTX was discontinued in one out of five patients after attaining CID in our cohort. However, approximately half of these patients flared after MTX withdrawal. In a large cohort of prospectively followed patient with JIA treated with MTX, we demonstrated that the flare risk was a function of time with CID before MTX discontinuation. The shorter the patient was in CID, the higher was the likelihood for disease flare. This finding is in contrast with the results of a randomised clinical trial (RCT) by Foell and colleagues.[17] Briefly summarised, patients with JIA were enrolled in the RCT at first confirmation of CID under MTX. Patients were randomised into two groups. One group was assigned to continue MTX treatment for 6 months in remission,

and the other one was assigned to continue MTX treatment for 12 months. In this study, the American College of Rheumatology (ACR) criteria for CID and remission on treatment were used, and a flare was defined as the presence of any disease activity indicator. Both groups exhibited an almost similar flare rate after MTX discontinuation. However, a total of 42 (23%) experienced a phase of active disease during MTX treatment on follow-up in the group that was assigned to continue MTX for 12 months; 19 patients (10.4%) in the 6-month treatment arm experienced a phase of active disease. In contrast, we selected the follow-up at which the paediatric rheumatologist discontinued MTX by attaining CID as index date. The time in CID before MTX discontinuation was the interval between the first visit with documentation of CID and continuous CID for subsequent visits up to the index date. Thus, the time of CID before MTX discontinuation was not interrupted in our study. We hypothesise that the remarkable proportion of patients with a phase of active disease before MTX discontinuation and the different study design may explain the divergent results between our study and the study of Foell *et al*[17] because the time in CID was shorter than 6 or 12 months for patients who flared in the RCT.

Furthermore, the considered categories of CID before MTX stop cannot be directly compared between the two studies. Patients discontinued MTX at month 6 and 12 after start of CID in the RCT,[17] whereas we considered time intervals of <6 months (mean 1.9 months in CID), 6–12 months (mean 8.5 months in CID) and more than 12 months (mean 16.5 months in CID).

The comparability of our study and the Foell *et al*'s study is limited by the different methods used to define CID and disease flare. Foell *et al* applied the Wallace criteria,[18] whereas our definition relies on the cJADAS10,[15] because of the non-availability of the report about uveitis activity for each visit. At the beginning of BiKeR in early 2001, the American College of Rheumatology pediatric response criteria (PedACR) criteria were used as outcome measure, the documentation of uveitis activity at each visit was added to the register later (and after publication of the Wallace criteria). Recently, a study comparing and discussing the different measures of CID in patients with JIA was published by Shoop-Worrall *et al*.[19] Shoop-Worrall *et al*[19] found the lowest rate for CID based on the cJADAS definition followed by the Wallace criteria. The cJADAS was often determined with values between 1 and 3, although the physician had rated the disease as inactive. This was due to the parental evaluation of general well-being. Given this observation, one may assume that we slightly underestimated the time in CID before MTX discontinuation and slightly overestimated the flare rate in follow-up.

A further explanation for the competing study result may be the difference in the patient cohorts in respect to the distribution of JIA categories. We included a remarkable higher proportion of patients with enthesitis-related arthritis and psoriatic arthritis, whereas we had less patients with RF-negative polyarthritis as compared with the study of Foell *et al*.[17] However, the mean disease duration at MTX withdrawal was comparable between the two studies.

Currently, no guidelines and consented recommendations exist for tapering or even withdrawing medication after attaining a state of CID in JIA. Tapering and withdrawing of medication is complex and should be based on the needs of individual patients.[20] One issue concerns the optimal time of stopping medication. There are reported inconsistent results in the literature, as discussed above. The majority of clinicians state that they wait 1–2 years before stopping the DMARD once the patient achieved clinical remission[20] rather than the 6-month window as noted in the trial of Foell *et al*.[17]

Oligoarthritis is supposedly regarded as the mildest JIA category with little or no damage to joints. In contrast, patients with oligoarthritis had significantly increased flare rates (67%) compared with other JIA categories. In addition, MTX treatment was initiated later in the disease course for patients with oligoarthritis (2.7 years vs 1.8 years for the other JIA categories). The treatment of oligoarthritis typically starts less aggressive. Then, NSAIDs and/or intra-articular glucocorticoid injections are escalated with MTX as soon as it becomes clear that the disease evolved to extended oligoarthritis.[10 21] The less aggressive treatment of oligoarthritis in routine care was also reflected by the shorter time under MTX before its discontinuation (8.1 months vs 13.1 months for the other JIA categories) in our registries. One could hypothesise that a treat to target concept based on more rapid therapy escalation in patients with a poor response to NSAIDs and glucocorticoids may improve the future outcome of oligoarthritis in these patients.

The limitations of our results should be noted. This is an observational study, and the individual decision for the discontinuation of MTX was made by the paediatric rheumatologist instead of by chance, as in a clinical trial. In our opinion, the potential bias of a non-randomised treatment decision is minimised by the large number of participating rheumatology centres and corresponding variation in treatment decisions. We did not collect data on the date when the patient achieved CID. The time in CID starts with the visit with the first documentation of a cJADAS10 less than or equal to one. Therefore, the estimated time in CID before MTX withdrawal may be underestimated in our study for some patients. A similar limitation exists for the time until recurrence of active disease after MTX withdrawal. The time until flare may be overestimated because we do not record the date of flare. The date of flare was the first physician visit when the patient has had at least moderate disease activity for the first time (cJADAS10 >1.5/2.5). The time until flare may be correctly determined in patients who required the start of DMARD treatment (63%), because the date of start of medication is reported by the physician. The flare rate may be slightly underestimated by the non-inclusion of start of NSAID and/or glucocorticoid treatment in the definition of recurrence of active disease, because the physician reports about NSAID and glucocorticoid use in the last 6 months at each visit without start and stop dates in JuMBO.

We observed a high flare rate in JIA after MTX discontinuation once the patients achieved CID. A flare was less common in patients who spent at least 12 months in CID before MTX discontinuation. Withdrawing treatment in JIA is complex, and currently no guidelines or consented recommendations have been published. The clinician must balance the high risk for disease flares that may prevent the clinician from withdrawing treatment against the risk for AEs and MTX intolerance under continuing treatment.

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Contributors All the authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All the authors substantially contributed to the study concept and design, analysis and interpretation of data, drafting of the manuscript and critical revision of the manuscript for important intellectual content. KM, MN and GH were responsible for the acquisition of data. Statistical analysis was done by JK. KM and GH obtained the funding for the study and supervised the study. Administrative, technical or material support was done by MN.

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Patient consent Obtained.

Ethics approval BiKeR was approved by the ethics committee of the Medical Council of North-Rhine Westfalia, Duesseldorf, Germany, and all patients provided informed consent. JuMBO was approved by the ethics committee of the Charité University Medicine Berlin. Young adults provided informed consent to participate in JuMBO.

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REFERENCES

1 Petty RE, Southwood TR, Manners P, *et al*. International league of associations for rheumatology classification of juvenile idiopathic arthritis: Second revision, Edmonton, 2001. *J Rheumatol* 2004;31:390–2.
2 Giannini EH, Brewer EJ, Kuzmina N, *et al*. Methotrexate in resistant juvenile rheumatoid arthritis. Results of the U.S.A.-U.S.S.R. double-blind, placebo-controlled trial. The Pediatric Rheumatology Collaborative Study Group and The Cooperative Children's Study Group. *N Engl J Med* 1992;326:1043–9.
3 Ruperto N, Murray KJ, Gerloni V, *et al*. A randomized trial of parenteral methotrexate comparing an intermediate dose with a higher dose in children with juvenile idiopathic arthritis who failed to respond to standard doses of methotrexate. *Arthritis Rheum* 2004;50:2191–201.
4 Albarouni M, Becker I, Horneff G. Predictors of response to methotrexate in juvenile idiopathic arthritis. *Pediatr Rheumatol Online J* 2014;12:35.
5 Klein A, Kaul I, Foeldvari I, *et al*. Efficacy and safety of oral and parenteral methotrexate therapy in children with juvenile idiopathic arthritis: an observational study with patients from the German Methotrexate Registry. *Arthritis Care Res* 2012;64:1349–56.
6 Bulatović M, Heijstek MW, Verkaaik M, *et al*. High prevalence of methotrexate intolerance in juvenile idiopathic arthritis: development and validation of a methotrexate intolerance severity score. *Arthritis Rheum* 2011;63:2007–13.
7 Ravelli A, Gerloni V, Corona F, *et al*. Oral versus intramuscular methotrexate in juvenile chronic arthritis. Italian Pediatric Rheumatology Study Group. *Clin Exp Rheumatol* 1998;16:181–3.
8 van der Meer A, Wulffraat NM, Prakken BJ, *et al*. Psychological side effects of MTX treatment in juvenile idiopathic arthritis: a pilot study. *Clin Exp Rheumatol* 2007;25:480–5.
9 Brunner HI, Johnson AL, Barron AC, *et al*. Gastrointestinal symptoms and their association with health-related quality of life of children with juvenile rheumatoid arthritis: validation of a gastrointestinal symptom questionnaire. *J Clin Rheumatol* 2005;11:194–204.
10 Beukelman T, Patkar NM, Saag KG, *et al*. 2011 American College of Rheumatology recommendations for the treatment of juvenile idiopathic arthritis: initiation and safety monitoring of therapeutic agents for the treatment of arthritis and systemic features. *Arthritis Care Res* 2011;63:465–82.
11 Horneff G. Update on biologicals for treatment of juvenile idiopathic arthritis. *Expert Opin Biol Ther* 2013;13:361–76.
12 Horneff G, Schmeling H, Biedermann T, *et al*. The German etanercept registry for treatment of juvenile idiopathic arthritis. *Ann Rheum Dis* 2004;63:1638–44.
13 Minden K, Niewerth M, Zink A, *et al*. Long-term outcome of patients with JIA treated with etanercept, results of the biologic register JuMBO. *Rheumatology* 2012;51:1407–15.
14 Petty RE, Southwood TR, Baum J, *et al*. Revision of the proposed classification criteria for juvenile idiopathic arthritis: Durban, 1997. *J Rheumatol* 1998;25:1991–4.
15 Consolaro A, Negro G, Chiara Gallo M, *et al*. Defining criteria for disease activity states in nonsystemic juvenile idiopathic arthritis based on a three-variable juvenile arthritis disease activity score. *Arthritis Care Res* 2014;66:1703–9.
16 Guzman J, Oen K, Tucker LB, *et al*. The outcomes of juvenile idiopathic arthritis in children managed with contemporary treatments: results from the ReACCh-Out cohort. *Ann Rheum Dis* 2015;74:1854–60.
17 Foell D, Wulffraat N, Wedderburn LR, *et al*. Methotrexate withdrawal at 6 vs 12 months in juvenile idiopathic arthritis in remission: a randomized clinical trial. *JAMA* 2010;303:1266–73.
18 Wallace CA, Giannini EH, Spalding SJ, *et al*. Clinically inactive disease in a cohort of children with new-onset polyarticular juvenile idiopathic arthritis treated with early aggressive therapy: time to achievement, total duration, and predictors. *J Rheumatol* 2014;41:1163–70.
19 Shoop-Worrall SJW, Verstappen SMM, Baildam E, *et al*. How common is clinically inactive disease in a prospective cohort of patients with juvenile idiopathic arthritis? The importance of definition. *Ann Rheum Dis* 2017;76:1381–8.
20 Broughton T, Armon K. Defining juvenile idiopathic arthritis remission and optimum time for disease-modifying anti-rheumatic drug withdrawal: why we need a consensus. *Paediatr Drugs* 2012;14:7–12.
21 Dueckers G, Guellac N, Arbogast M, *et al*. Evidence and consensus based GKJR guidelines for the treatment of juvenile idiopathic arthritis. *Clin Immunol* 2012;142:176–93.

EXTENDED REPORT

ADJUVITE: a double-blind, randomised, placebo-controlled trial of adalimumab in early onset, chronic, juvenile idiopathic arthritis-associated anterior uveitis

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ABSTRACT

Objectives To assess the efficacy and safety of adalimumab on uveitis in patients with early onset, chronic, juvenile idiopathic arthritis (JIA)-associated or idiopathic anterior uveitis and an inadequate response to topical steroids and methotrexate (MTX).

Methods Patients aged 4 years or more with ocular inflammation quantified by laser flare photometry (LFP) ≥30 photon units/ms were double-blindly randomised (1:1) to 2 groups, one treated with placebo and one with adalimumab subcutaneously at a dose of 24 mg/m^2 in patients aged <13 years, 40 mg in the others, every other week. The primary outcome was response at month 2 (M2) defined as a 30% reduction of inflammation on LFP in the assessable eye with more severe baseline inflammation and no worsening on slit lamp examination. From M2 to M12, all patients received adalimumab.

Results At M2, among 31 patients included in intention-to-treat analysis, there were 9/16 responders on adalimumab and 3/15 on placebo (P=0.038, X^2 test; relative risk=2.81, 95% CI 0.94 to 8.45; risk difference: 36.3%, 95% CI 2.1 to 60.6); there was no significant difference using the Standardised Uveitis Nomenclature classification criteria of improvement. Thirty patients continued the trial after M2 and received adalimumab (open-label phase), 29 reached M12. There were seven serious adverse events none related to study treatment.

Conclusions This trial is in favour of using adalimumab in patients with early onset, chronic anterior uveitis, which is in most cases associated with JIA, in case of inadequate response to topical therapy and MTX. LFP could be a valuable tool to assess early treatment efficacy.

Trial registration number NCT01385826.

INTRODUCTION

More than 20% of children with early onset (usually before 6 years), rheumatoid factor negative polyarticular or oligoarticular juvenile idiopathic arthritis (JIA), and some children without arthritis, most of them with non-specific antinuclear antibodies develop chronic, remitting, anterior uveitis.[1] Most of them require long-lasting local steroid treatment or even oral and pulsed intravenous steroids. Severe complications develop in many cases due to long-lasting ocular inflammation and steroid treatment.[2–7] Treatment with methotrexate (MTX) has been reported with an improvement of uveitis and a reduction of the number of flares[8–11]; however, many patients do not respond adequately.[12] Adalimumab is an antitumour necrosis factor-α (anti-TNF-α) antibody that demonstrated its efficacy on arthritis, alone or in association with MTX, in children with JIA and a polyarticular course in the absence of active uveitis.[13] In patients with JIA-associated uveitis and an inadequate response to MTX and topical steroids, several uncontrolled studies are in favour of using adalimumab.[14–25] In such patients, the randomised placebo-controlled SYCAMORE trial recently demonstrated that adding adalimumab to MTX was effective in preventing treatment failure.[26] The assessment of ocular inflammation was based on grading the cellularity of the anterior chamber of the eye by slit lamp (SL), using definitions of improvement or worsening according to the Standardised Uveitis Nomenclature (SUN) classification.[27] Treatment failure was defined by a set of criteria that included presence on two consecutive assessments of high-grade uveitis activity at 3 months or later, uveitis worsening, new-onset or worsening of uveitis complication. Patients were followed until treatment failure or for 18 months. Treatment failure was observed in 16 out of 60 patients on adalimumab versus 18 out of 30 on placebo, which was highly significant.

Grading uveitis activity by SL may lack sensitivity to document improvement, particularly in patients with chronic active uveitis but low cell counts. Laser flare photometry (LFP), which measures the anterior chamber protein flare in an objective and sensitive manner, has previously shown its reliability to assess the degree of ocular inflammation, the prognosis of uveitis and the effect of treatments.[28–33]

Although neither the SUN criteria nor LFP has been validated in paediatric uveitis, experts are in favour of using the SUN criteria as an important outcome measure and consider LFP as a tool for clinical trials.[33 34] Based on preliminary experience of ophthalmologists, we designed a double-blind randomised trial, which objective was to assess adalimumab efficacy versus placebo on uveitis over a period of 2 months by using LFP in addition to SL examination in patients with early onset, chronic anterior uveitis and an inadequate response to topical steroids and MTX.

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METHODS

Study design and participants

This was a double-blind, 1:1 randomised, placebo-controlled multicentre phase III trial that aimed at assessing the efficacy of 2 months of adalimumab treatment, with then an open-label period where all patients were followed up under adalimumab for an extra 10 months.

The trial involved nine French tertiary care centres for paediatric rheumatology and seven associated ophthalmologic centres experienced in using LFP in children.

An independent data and safety monitoring board was appointed for this trial. The study is registered at ClinicalTrials.gov under number NCT01385826.

Eligibility criteria are listed in online supplementary table S1. Patients aged at least 4 years had chronic, active anterior uveitis (defined by an inflammation of at least 30 ph/ms quantified with LFP) associated with JIA or idiopathic, and an inadequate response to well-conducted topical steroid therapy and MTX at a dose of 0.3–0.6 mg/kg (without exceeding 25 mg) once a week for at least 3 months.

Major exclusion criteria were: systemic-onset, rheumatoid factor positive or enthesitis-related JIA, previous treatment with anti-TNF monoclonal antibody, any contraindication to administration of immunosuppressive therapy, complications requiring surgery.

Treatment and procedures

An initial screening visit aimed to assess patient eligibility and present the study to the patient and its parents. After signing the informed consent form, patient was examined by an investigator ophthalmologist and a rheumatologist or paediatrician experienced in paediatric rheumatology, then biologic tests and other investigations required to confirm eligibility were performed (see online supplementary table S2). Inclusion visit could occurred from 2 days to 1 month later. When inclusion was confirmed, patients were randomly assigned (1:1) to receive either adalimumab or placebo. Patients on MTX were maintained on stable dose. The random assignment sequence stratified on age (<13 or ≥13 years) and using blocks of size four was computer-generated at Paris Descartes Clinical Research Unit. Patient received placebo or adalimumab at a dose of 24 mg/m^2 in patients aged <13 years, 40 mg in patients aged ≥13 years, every other week subcutaneous injections. Blinded injections were realised in consultation at day (D)0, D14, month (M)1 visit and at home at D42.

The double-blind phase lasted from D0 until M2, except in the case of dropout from the trial. From visit M2, all patients who continued the trial received adalimumab treatment during 10 months (open-labelled phase). Visits were held at M3, M4, M6, M9 and M12.

At each visit, there was an assessment by two investigators, first an ophthalmologist, then a paediatrician or a rheumatologist, to check the absence of contraindication to maintain the patient in the trial and to assess treatment tolerance. These two assessors were blinded to each other following D0 visit and the first injection of study treatment. Particular attention was demanded for patients who presented a significant worsening of ophthalmological condition or any other condition that justified rescue therapy.

Online supplementary table S2 details the clinical assessment performed by the ophthalmologist and an investigator paediatrician or rheumatologist, the assessment of JIA response or flare using the JIA American College of Rheumatology (ACR) core-set criteria[35 36] and the laboratory tests.

Primary and secondary outcomes

The primary outcome was response to treatment at the end of double-blind period (M2), defined as a reduction of at least 30% of ocular inflammation quantified by LFP without worsening of cell counts or protein flare on SL examination according to SUN criteria, in the assessable eye with more severe baseline inflammation. The method of analysis by LFP is described in online supplementary table S3.

Secondary outcomes included assessment of treatment efficacy on ocular inflammation quantified by SL examination and LFP at each visit (using 30%, 50% and 70% threshold to define response), modifications of the dose of topical and/or systemic steroid therapy between M2 and M12, JIA response or flare using the paediatric ACR core-set criteria, assessment of treatment safety at M2 and until M12, with adverse events (AE) reported according to the MedDRA dictionary and a peculiar attention to any AE of special interest, such as neoplasia, opportunist infection or occurrence of a new inflammatory disease.

At each visit, both the eye of the primary outcome and the fellow eye were evaluated.

Statistical analysis

Our study was designed to detect a response rate to treatment 40 percentage points upper in adalimumab group at the end of double-blind period (ie, equal to 50%), assuming a response of 10% under placebo. With a two-sided alpha risk equal to 5% and 80% power, 19 patients randomised per group were required. A total enrolment of 40 patients was thus planned.

All statistical analyses were undertaken using R V.2.11.1 software, and in accordance with the statistical analysis plan prespecified before the lock of the database. Statistical tests were two-sided and P values <0.05 were considered statistically significant. Baseline characteristics of the two groups were described as median (range) for quantitative variables and frequencies (%) for qualitative variables.

Our intention-to-treat (ITT) population consisted in all randomised participants who received at least one injection of trial medication. A per-protocol population was also defined, including all randomised patients without violation of eligibility criteria, who properly had their planned injections until the end of double-blind period and whose primary outcome is evaluable.

Primary outcome was compared between groups using X^2 test. Primary analysis was done on the ITT population, with patients who prematurely ended double-blind period or non-assessable patients considered as non-responders. No other imputation method of missing data was considered due to the small sample size. Treatment effect size was presented as a relative risk and 95% CI using a log-binomial regression model. To investigate the consistency of results, an analysis on per-protocol population was also performed.

Secondary outcomes and safety data related to the double-blind period were compared between groups using X^2 test (or Fisher's exact test when it was appropriate) and Student's t-test (or Wilcoxon test for non-normally distributed variables). No formal adjustment was made to any P values to allow for the large number of secondary end points analysed, and thus P values for secondary analyses need to be interpreted conservatively. Secondary outcomes and safety data related to the open-label period were not compared but only described globally and according to previous allocated group in all patients from their first administration of open-label treatment.

RESULTS

From June 2011 to August 2014, 32 patients were randomised in five centres. Enrolment was slower than anticipated and we could not extend the trial duration to reach the number of patients expected.

All but one patient received at least one study treatment injection; the latter withdrew from study before the first administration and was thus excluded from ITT population. Two other patients were included with baseline LFP value inferior to 30 ph/ms, hence not respecting one of the inclusion criteria, both in the adalimumab arm; they were excluded from per-protocol population (figure 1). Patients baseline characteristics are shown in table 1. Some differences between both group can be noticed such as the proportion of patients with bilateral uveitis, cataract or on MTX treatment at baseline.

Double-blind period (D0-M2)

Five patients prematurely ended double-blind period, one in the adalimumab arm, four in the placebo arm (Figure 1). Four of these five patients had a premature M2 visit but continued the open-label phase of trial under adalimumab afterwards. One patient from the placebo arm, with a past history of ocular hypertonia definitively ended the trial at D14 for marked hypertonia that was documented as a serious AE (SAE).

For primary outcome analysis, as shown in table 2, there were in ITT 9/16 responders (56%) in the adalimumab arm and 3/15 (20%) in the placebo arm (P=0.038, X^2 test; relative risk (RR)=2.81 (95% CI 0.94 to 8.45)). As two non-responders from adalimumab arm had violation of inclusion criteria (baseline LFP value <30 ph/ms), in per-protocol population, there were 9/14 responders (64%) on adalimumab and 3/15 responders (20%) on placebo (P=0.015, X^2 test; RR=3.21 (95% CI 1.09 to 9.51)).

Changes in topical steroid therapy in the eye evaluated for the primary outcome was documented in four patients from screening to M2. In two responders on placebo, the number of eye drops was increased from 1 to 3 and 5 drops, respectively at or before screening visit. In a non-responder patient on adalimumab, the number of drops had been decreased from 3 to 2 after screening visit. Finally, in one non-responder patient on placebo the number of eye drops had been decreased from 3 to 2 in both eyes between D14 and M1.

Among M2 responders, two patients were responders from D14 (one patient on adalimumab, one on placebo) and six from M1 (five on adalimumab, one patient on placebo). Fifty per cent improvement on LFP from baseline was documented at M1 in two patients on adalimumab, at M2 in four patients on adalimumab and two on placebo; 70% improvement was documented at M2 in three patients on adalimumab.

Worsening in SL cellular as defined by the SUN occurred in one patient from placebo group (who was non-responder according to LFP measurements) at M2. There was no significant difference between both arms regarding the proportion of patients who disclosed improvement according to the SUN criteria from D0 to M2 in SL cellular or protein grading (table 2).

A JIA flare (ACR 70 criteria) was observed in one patient under placebo at D14; there was no significant change in this patient regarding ophthalmologic assessment.

Open-label phase (M2-M12)

Among 30 patients who entered the open-label phase of trial after M2 visit, one discontinued 5.8 months later due to a flare of both uveitis and arthritis; the other 29 patients reached M12 under adalimumab.

Figure 2A,B show at each visit and for each patient the changes of anterior chamber flare measured by LFP. Among 45 eyes with LFP >10 ph/ms at M2, 36 were assessable at M12 and 17 had improved by 30% or more compared with M2. Online supplementary table S4 indicates the main changes regarding outcome measures and steroid therapy at M3 in patients who had been on adalimumab since D0 and at M12.

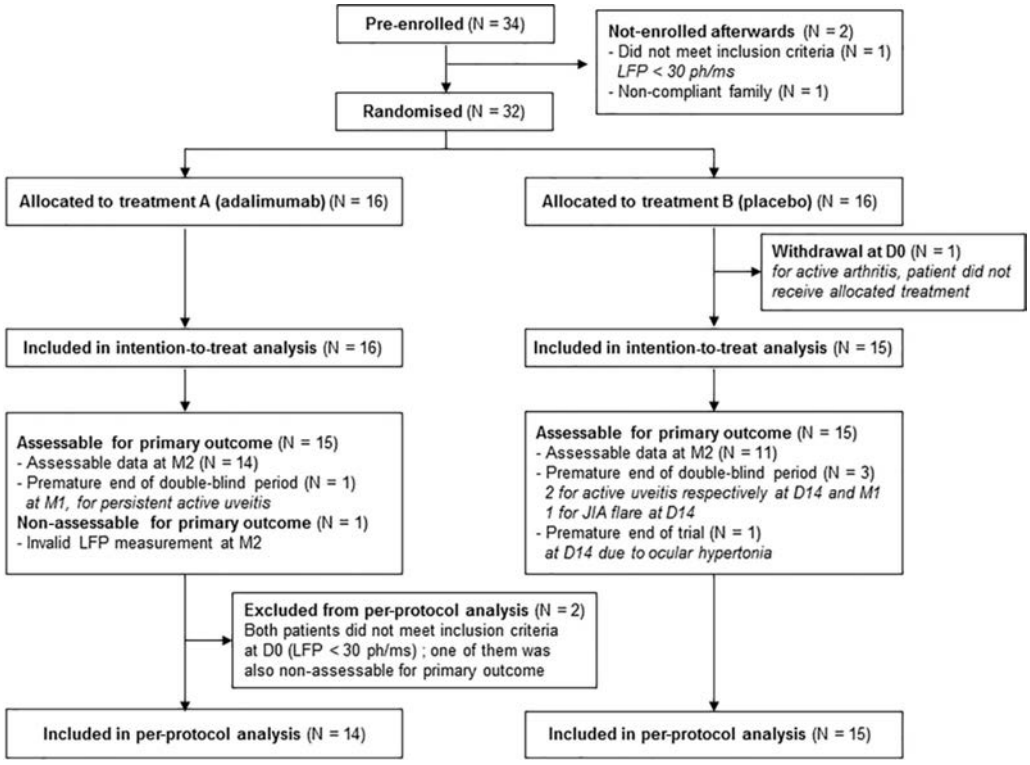

Figure 1 Patients flow chart. D, day; JIA, juvenile idiopathic arthritis; LFP, laser flare photometry ; M, month.

Table 1 Patients characteristics at study treatment onset

	Adalimumab (n=16)	Placebo (n=15)	All patients (n=31)
Demographic features			
Female, n (%)	15 (94)	13 (87)	28 (90)
Age, years, median (range)	10.8 (5.0–20.3)	9.2 (4.9–29.1)	9.5 (4.9–29.1)
Patients <13 years, n (%)	12 (75)	12 (80)	24 (77)
Weight, kg, median (range)	32.7 (17.9–74)	29.5 (-62.2)	31.6 (17.9–74)
Juvenile idiopathic arthritis (JIA) features			
JIA duration, years, median (range)	6.0 (1.8–12.6)	5.5 (0.4–27.9)	5.6 (0.4–27.9)
Oligoarticular-onset JIA, n (%)	12 (75)	13 (87)	25 (81)
Others JIA categories, n (%)	2* (13)	2* (13)	4* (13)
Positive antinuclear antibodies	14 (88)	13 (87)	27 (87)
Chronic uveitis, no arthritis, n (%)	2 (13)	0 (0)	2 (6)
No. of active joints, median (range)	0 (0–3)	0 (0–4)	0 (0–4)
No. of joints with LOM, median (range)	0 (0–9)	0 (0–1)	0 (0–9)
CHAQ, median (range)	0 (0–1)	0 (0–0.625)	0 (0–1)
Physician VAS, median (range)	0 (0–45)	0 (0–15)	0 (0–45
Patient/parent VAS, median (range)	0 (0–14)	0 (0–48)	0 (0–48)
ESR, median (range)	5.5 (2.0–15.0)	6.5 (2-30)	6.0 (2-30)
CRP, median (range)	6 (1–30.0)	6 (1-6)	6 (1-30)
Ophthalmologic features			
Uveitis median duration, years (range)	4.4 (0.4–8.9)	4.8 (0.6–24.2)	4.7 (0.4–24.2)
Bilateral uveitis, patients n (%)	10 (67)	14 (93)	24 (80)
Band keratopathy, patients n (%)	13/15 (87)	10 (67)	23/30 (77)
Cataract, patients n (%)	9/11 (82)	5/13 (38)	14/25 (56)
Posterior synechiae, patients n (%)	12/14 (86)	10/15 (67)	22/29 (76)
Vitritis, patients n (%)	8/15 (53)	9/15 (60)	17/30 (57)
Macular oedema*, patients n (%)	8/15(53)	6/15 (40)	14/30 (47)
Visual acuity†, median (range)	0.1 [–0.2–1.3)	0.0 (–0.2–1.0)	0.1 (–0.2–1.3)
Main characteristics of the eye evaluated for the primary outcome			
Laser flare (ph/ms), median (range)	99 (23–322)	70 (36–265)	73 (23–322)
Slit lamp protein			
SUN 0, patients n (%)	3 (19)	1 (7)	4 (13)
SUN 1, patients n (%)	5 (31)	5 (33)	10 (32)
SUN 2, patients n (%)	7 (44)	8 (53)	15 (48)
SUN 3, patients n (%)	1 (6)	1 (7)	2 (6)
Slit lamp cells			
SUN 0, patients n (%)	3 (19)	3 (20)	6 (19)
SUN 0.5, patients n (%)	5 (31)	4 (27)	9 (29)
SUN 1, patients n (%)	7 (44)	3 (20)	10 (32)
SUN 2, patients n (%)	1 (6)	4 (27)	5 (16)
SUN 3, patients n (%)	0 (0)	1 (7)	1 (3)
Previous treatments			
Oral steroids, patients n (%)	9 (56)	8 (53)	17 (55)
MTX, patients n (%)	16 (100)	15 (100)	31 (100)
Etanercept, patients n (%)	2 (13)	2 (13)	4 (13)
Ongoing treatments at D0 (median doses and range for patients on treatment)			
Patients on steroids eye drops‡, n (%)	12 (75)	10 (67)	22 (71)
Dexamethasone eye drops, n (range)‡	3 (1-6)	3 (1-5)=	3 (1-6)
Oral steroids, patients n (%)	7 (44)	3 (20)	10 (32)
Predn. daily dose (mg), median (range)	5 (1-12)	2 (2-15)	5 (1-15)
MTX, patients n (%)	15 (94)	11 (73)	26 (84)
MTX dose in mg/week, median (range)	12.5 (5-20)	12.5 (7.5–17.5)	12.5 (5-20)
MTX in mg/m^2/week, median (range)	11.3 (3.4–14.5)	12.3 (7.2–15.8)	11.5 (3.4–15.8)

*Two cases of rheumatoid factor negative polyarticular JIA in the adalimumab group, two cases of unclassified JIA in the placebo group.
†Macular thickness was measured by optical coherence tomography and values superior to 320 microns were considered sufficient to confirm macular oedema.
‡Expressed in log (MAR), most of patients were evaluated using ETDRS charts but Monoyer and Snellen charts had also been used in a few cases.
§Eye evaluated for the primary outcome.
CHAQ, Childhood Health Assessment Questionnaire; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; JIA, juvenile idiopathic arthritis; LOM, joints with limitation of motion; MTX, methotrexate; n, number; Predn., prednisone or prednisolone; SUN, Standardised Uveitis Nomenclature; VAS, visual analogue scale (from 0 to 100) allowing to measure JIA disease activity.

Table 2 Responses at month 2 (end of double-blind period)

	Response rate (%)				
	Adalimumab	Placebo	P value	RR (95% CI)	Risk difference (95% CI)
LFP improvement ≥30% and no worsening on slit lamp					
ITT population (primary outcome)	9/16 (56)	3/15 (20)	0.038	2.81 (0.94 to 8.45)	+36.3% (2.1 to 60.6)
Per-protocol population	9/14 (64)	3/15 (20)	0.015	3.21 (1.09 to 9.51)	+44.3% (8.4 to 67.6)
All eyes with D0 LFP >30 ph/ms	9/17 (53)	4/22 (18)	0.022	2.91 (1.08 to 7.86)	+34.8% (4.8 to 58.3)
Slit lamp improvement (SUN criteria), eyes of the primary outcome					
Cell counts	2/16 (13)	3/15 (20)*	0.65		
Proteins	5/16 (31)	5/15 (33)	1		
LFP median (min-max) evolution (%) from D0 to M2					
Eyes of the primary outcome	−32 (−82 to +35)	−4 (−57 to +14)	0.20		
All eyes with D0 LFP >30 ph/ms	−32 (−82 to +35)	−5 (−57 to +59)	0.03		
Slit lamp cellular score (SUN criteria) at M2, eyes of the primary outcome					
Grade 0	4/16†	4/15†	0.69		
Grade 0.5	9/16	7/15	NS		
Grade 1	3/16	2/15	NS		
Grade 2	0/16	2/15*	NS		
Visual acuity‡, median (range)	0.15 (−0.10 to 1.10)	0.00 (−0.10 to 0.60)			
Macular oedema§, number of cases	3/16	3/14¶			

*Significant worsening was documented in one case in the placebo group.
†No patient had LFP values <10 ph/ms; in the patients with cellular SUN grade 0 at M2, LFP values ranged from 23 to 315 mh/ms.
‡Expressed in log (MAR).
§Macular thickness was measured by optical coherence tomography and values superior to 320 microns were considered sufficient to confirm macular oedema.
¶One eye was not assessable at M2.
D0, day 0 (baseline); ITT, intention-to-treat; LFP, laser flare photometry; RR, relative risk; SUN, Standardised Uveitis Nomenclature.

A JIA flare (ACR 70 criteria) was observed at M12 in one patient.

Safety

In addition to the SAE that occurred during the double-blind phase, six SAEs occurred during the open-labelled phase, all in patients who were initially randomised to placebo. No SAE was related to study treatment and their outcome was favourable in all cases. Regarding non-SAEs, there were equally distributed between both groups during the double-blind phase and no event of special interest was reported (table 3 and supplementary table S5).

DISCUSSION

In patients with early onset idiopathic or JIA-associated chronic uveitis and an inadequate response to topical steroids and MTX, 2 months of treatment with adalimumab was effective in reducing ocular inflammation compared with placebo, as assessed by 30% improvement or more of anterior chamber inflammation on LFP and improvement or stable appearance on SL examination. Over 12 months, all patients but two continued adalimumab, which was well tolerated and associated with persistent uveitis improvement in most cases.

The result of the primary end point should be interpreted with caution given the small size of this study. This is also reflected by the wide CI RR which contains 1. However, this CI is narrower when recalculated with a more appropriate method for small samples (Koopman asymptotic score),[37 38] RR=2.81 (1.05–8.50).

Combining LFP to SL examination, we were able to document early improvement on adalimumab. This would not have been feasible using only SL examination based on SUN criteria in our population of patients who had in most cases low or relatively low cell counts, as can be expected nowadays for patients carefully followed by their ophthalmologist. On the other hand, only few ophthalmologists were equipped with LFP and experienced using it in children when we started the trial, which limited both the number of participating centres and the number of patients we were able to enrol, hence reducing the power to detect a difference between groups. Proposing a 30% decrease of anterior chamber flare to define significant LFP improvement was based on the experience of expert ophthalmologists and ended up being a reasonable target, even if more patients on placebo than expected reached it. Interestingly, the observation that patients with very high LFP could be quick responders but that some patients with relatively mild inflammation on LFP at study entry needed more than 2 months to reach 30% improvement suggests that a longer period could be required to document significant improvement in such patients.

The proportion of M2 responders may have been influenced in favour of the placebo arm by a marked increase in the number of drops at or before screening visit in the eye of the primary outcome in two patients from the placebo group who responded from D14. Although clinically justified from an ophthalmologic point of view, such significant modifications of local therapy most likely negatively affected the trial results.

The latest follow-up data are encouraging as 29 out of 31 patients were still on adalimumab and most had no more inflammation or less inflammation than at study entry. In addition, most patients on oral steroids had stopped or decreased this treatment. Also, most of patients had stopped local treatment or decreased the number of drops between M2 and M12. This is particularly important as persistent, long-lasting inflammation and local steroids therapy are associated with a high risk of complications in young adults.[2–7]

Safety data were reassuring as there was no SAE or AE of special interest that were related to adalimumab therapy, most SAEs being linked to the underlying disease and to complications that had developed before trial onset, particularly ocular hypertonia. Therefore, starting adalimumab at an earlier stage

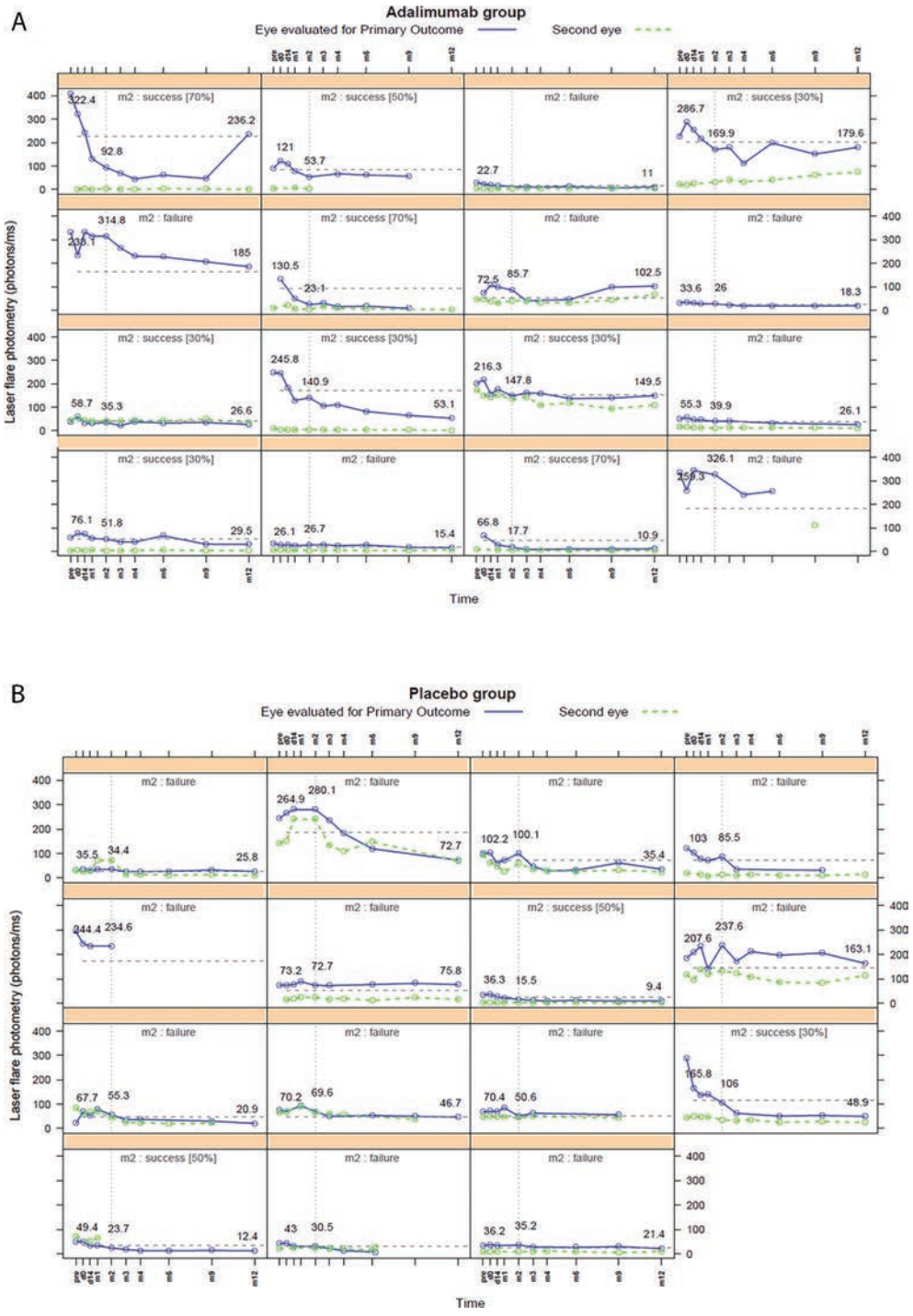

Figure 2 Values of the anterior chamber flare measured by laser flare photometry (ph/ms) visit after visit in each patient; (A) patients of the adalimumab group and (B) patients of the placebo group. In blue, eye of the primary outcome and in dotted green the fellow eye. The dotted vertical line indicates the M2 visit (primary end point visit) and the dotted horizontal line indicates for each patient the threshold of 30% improvement compared with baseline (day 0) visit for the eye of the primary outcome.

might be worth considering in the future. However, this was a small series with a relatively short follow-up and the risk of AEs including severe infections, although relatively low, has been well documented in children as in adults on anti-TNF-α treatment.[39]

Our results confirm several observational series of patients, who showed that anti-TNF-α antibodies, could be associated with a reduction of inflammation and of the number of uveitis flare.[14–25] While the SYCAMORE trial aimed to demonstrate that adalimumab could prevent treatment failure, and indeed demonstrated in a double-blind randomised fashion in a large number of patients that adalimumab was superior to placebo in either reducing inflammation or avoid uveitis worsening within 3 months and well tolerated over 12 months,[26] ADJUVITE aimed to demonstrate early uveitis improvement on adalimumab. In addition to conforming the results of SYCAMORE on several common outcome measures, ADJUVITE showed that LFP assessment, which detects inflammation even in patients with low-grade inflammation and sometimes no cells on SL examination as shown by response to maximised therapy,[33] enables an earlier documentation of improvement on adalimumab therapy.

Table 3 Adverse events

	Part A (D0-M2, double-blind)				Part B (M2-12)	
	Group 1		Group 2		(open label)	
	Adalimumab (n=16)		Placebo (n=15)		Adalimumab (n=30)	
	No. of events	No. (%) of patients	No. of events	No. (%) of patients	No. of events	No. (%) of patients
Serious adverse events	0	0	1	1	6	5*
Eye disorder						
Cataract	0	0	0	0	1	1 (3.3)
Ocular hypertonia*	0	0	1	1 (6.7)	2	2 (6.7)
Infection or infestation						
Tonsillitis	0	0	0	0	1	1 (3.3)
Investigation						
ALAT and ASAT increased	0	0	0	0	2	2 (6.7)†
Non-serious adverse events observed in at least two patients, and ophthalmological events						
Blood or lymphatic system disorder						
Cervical adenitis	1	1 (6.2)	0	0	1	1 (3.3)
Eye disorder						
Eye inflammation	1	1 (6.2)	0	0	1	1 (3.3)
Macular oedema	0	0	0	0	1‡	1‡ (3.3)
Ocular hypertonia	1	1 (6.2)	0	0	1	1 (6.7)
Photophobia	0	0	0	0	1	1 (3.3)
Gastrointestinal disorder						
Abdominal pain	1	1 (6.2)	1	1 (6.7)	4	3 (10)
Nausea	1	1 (6.2)	1	1 (6.7)	2	2 (6.7)
Vomiting	1	1 (6.2)	0	0	2	2 (6.7)
General disorder or injection site condition						
Injection site pain	1	1 (6.2)	1	1 (6.7)	2	2 (6.7)
Pain	0	0	1	1 (6.7)	2	2 (6.7)
Fever	2	2 (12.5)	1	1 (6.7)	4	3 (10)
Infection or infestation						
Bronchitis	0	0	0	0	3	2 (6.7)
Conjunctivitis	0	0	1	1 (6.7)	1	1 (3.3)
Impetigo	1	1 (6.2)	0	0	1	1 (3.3)
Molluscum contagiosum	1	1 (6.2)	0	0	1	1 (3.3)
Otitis	2	2 (12.5)	0	0	2	2 (6.7)
Rhinitis, pharyngitis, tracheitis	2	2 (12.5)	6	5 (33.3)	16	13 (43.3)
Tonsillitis	0	0	1	1 (6.7)	3	2 (6.7)
Musculoskeletal and connective tissue disorder						
Arthralgia	0	0	2	2 (13.3)	7	4 (13.3)
Arthritis	0	0	§	§	2	2 (6.7)
Myalgia	0	0	2	1 (6.7)	0	0
Neoplasm benign and malignant						
Planar warfs	0	0	0	0	2	2 (6.7)
Nervous system disorder						
Headache	0	0	0	0	4	3 (10)
Respiratory, thoracic and mediastinal disorder						
Cough	0	0	1	1 (6.7)	3	3 (10)
Skin and subcutaneous tissue disorder						
Skin eruption	1	1 (6.2)	1	1 (6.7)	2	2 (6.7)

*Ocular hypertonia was the source of serious adverse event in three patients; it occurred during the double-blind period in one patient who was withdrawn from the trial at M1 visit, during the open-labelled phase in two patients, one of which required trabeculectomy 5.4 months after inclusion.
†Transient, resolved in both cases after methotrexate withdrawal.
‡This patient, who had developed macular oedema in the past.
§One patient had a JIA flare that led to premature withdrawal from the double-blind phase but was not documented as an adverse event.
¶Two serious adverse events occurred in the same patient (transient hepatic cytolysis 10.6 months after inclusion and ocular hypertension 11.3 months after inclusion).
**Was randomised to the placebo group and was non-responder for the primary objective. At M6 visit, after 4 months of adalimumab and while the dose of oral steroids had been tapered, macular oedema reappeared, oral steroid dosage was then increased, further ophthalmologic assessments were reassuring and the dose of oral steroids was again reduced at M9 visit.
ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; D, day; JIA, juvenile idiopathic arthritis; M, month.

This could help to decide after a few months (2 months in the patients with high LFP values at treatment onset, possibly more in the other patients), if adalimumab therapy should be continued or other treatments discussed.

In conclusion, this trial is in favour of using adalimumab in patients with early onset, chronic anterior uveitis, which is in most cases associated with JIA, in case of inadequate response to topical therapy and MTX. It also suggests that LFP could be a valuable tool to assess early treatment efficacy.

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REFERENCES

1 Ravelli A, Felici E, Magni-Manzoni S, *et al*. Patients with antinuclear antibody-positive juvenile idiopathic arthritis constitute a homogeneous subgroup irrespective of the course of joint disease. *Arthritis Rheum* 2005;52:826–32.
2 Haasnoot AJ, Vernie LA, Rothova A, *et al*. Impact of juvenile idiopathic arthritis associated uveitis in early adulthood. *PLoS One* 2016;11:e0164312.
3 Böhm MR, Tappeiner C, Breitbach MA, *et al*. Ocular hypotony in patients with juvenile idiopathic arthritis-associated uveitis. *Am J Ophthalmol* 2017;173:45–55.
4 Oray M, Khachatryan N, Ebrahimiadib N, *et al*. Ocular morbidities of juvenile idiopathic arthritis-associated uveitis in adulthood: results from a tertiary center study. *Graefes Arch Clin Exp Ophthalmol* 2016;254:1841–9.
5 Stroh IG, Moradi A, Burkholder BM, *et al*. Occurrence of and risk factors for ocular hypertension and secondary glaucoma in juvenile idiopathic arthritis-associated uveitis. *Ocul Immunol Inflamm* 2017;25:503–12.
6 Marvillet I, Terrada C, Quartier P, *et al*. Ocular threat in juvenile idiopathic arthritis. *Joint Bone Spine* 2009;76:383–8.
7 Kotaniemi K, Arkela-Kautiainen M, Haapasaari J, *et al*. Uveitis in young adults with juvenile idiopathic arthritis: a clinical evaluation of 123 patients. *Ann Rheum Dis* 2005;64:871–4.
8 Foeldvari I, Becker I, Horneff G. Uveitis events during adalimumab, etanercept, and methotrexate therapy in juvenile idiopathic arthritis: data from the biologics in pediatric rheumatology registry. *Arthritis Care Res* 2015;67:1529–35.
9 Papadopoulou C, Kostik M, Böhm M, *et al*. Methotrexate therapy may prevent the onset of uveitis in juvenile idiopathic arthritis. *J Pediatr* 2013;163:879–84.
10 Foeldvari I, Wierk A. Methotrexate is an effective treatment for chronic uveitis associated with juvenile idiopathic arthritis. *J Rheumatol* 2005;32:362–5.
11 Simonini G, Paudyal P, Jones GT, *et al*. Current evidence of methotrexate efficacy in childhood chronic uveitis: a systematic review and meta-analysis approach. *Rheumatology* 2013;52:825–31.
12 Heiligenhaus A, Mingels A, Heinz C, *et al*. Methotrexate for uveitis associated with juvenile idiopathic arthritis: value and requirement for additional anti-inflammatory medication. *Eur J Ophthalmol* 2007;17:743–8.
13 Lovell DJ, Ruperto N, Goodman S, *et al*. Adalimumab with or without methotrexate in juvenile rheumatoid arthritis. *N Engl J Med* 2008;359:810–20.
14 Kotaniemi K, Säilä H, Kautiainen H. Long-term efficacy of adalimumab in the treatment of uveitis associated with juvenile idiopathic arthritis. *Clin Ophthalmol* 2011;5:1425–9.
15 Tynjälä P, Kotaniemi K, Lindahl P, *et al*. Adalimumab in juvenile idiopathic arthritis-associated chronic anterior uveitis. *Rheumatology* 2008;47:339–44.
16 Biester S, Deuter C, Michels H, *et al*. Adalimumab in the therapy of uveitis in childhood. *Br J Ophthalmol* 2007;91:319–24.
17 Foeldvari I, Nielsen S, Kümmerle-Deschner J, *et al*. Tumor necrosis factor-alpha blocker in treatment of juvenile idiopathic arthritis-associated uveitis refractory to second-line agents: results of a multinational survey. *J Rheumatol* 2007;34:1146–50.
18 Vazquez-Cobian LB, Flynn T, Lehman TJ. Adalimumab therapy for childhood uveitis. *J Pediatr* 2006;149:572–5.
19 Muñoz-Gallego A, Barral E, Enríquez E, *et al*. Adalimumab for the treatment of refractory noninfectious paediatric uveitis. *Int Ophthalmol* 2017;37:719–25.
20 Castiblanco C, Meese H, Foster CS. Treatment of pediatric uveitis with adalimumab: the MERSI experience. *J Aapos* 2016;20:145–7.
21 La Torre F, Cattalini M, Teruzzi B, *et al*. Efficacy of adalimumab in young children with juvenile idiopathic arthritis and chronic uveitis: a case series. *BMC Res Notes* 2014;7:316.
22 García-De-Vicuña C, Díaz-Llopis M, Salom D, *et al*. Usefulness of adalimumab in the treatment of refractory uveitis associated with juvenile idiopathic arthritis. *Mediators Inflamm* 2013;2013:1–6.
23 Bravo-Ljubetic L, Peralta-Calvo J, Noval S, *et al*. Adalimumab therapy for refractory childhood uveitis. *J Aapos* 2013;17:456–9.
24 Magli A, Forte R, Navarro P, *et al*. Adalimumab for juvenile idiopathic arthritis-associated uveitis. *Graefes Arch Clin Exp Ophthalmol* 2013;251:1601–6.
25 Zannin ME, Birolo C, Gerloni VM, *et al*. Safety and efficacy of infliximab and adalimumab for refractory uveitis in juvenile idiopathic arthritis: 1-year followup data from the Italian Registry. *J Rheumatol* 2013;40:74–9.
26 Ramanan AV, Dick AD, Jones AP, *et al*. Adalimumab plus Methotrexate for Uveitis in Juvenile Idiopathic Arthritis. *N Engl J Med* 2017;376:1637–46.
27 Jabs DA, Nussenblatt RB, Rosenbaum JT. Standardization of Uveitis Nomenclature (SUN) Working Group. Standardization of uveitis nomenclature for reporting clinical data. results of the first international workshop. *Am J Ophthalmol* 2005.

28 Ladas JG, Wheeler NC, Morhun PJ, *et al*. Laser flare-cell photometry: methodology and clinical applications. *Surv Ophthalmol* 2005;50:27–47.
29 Herbort CP, Guex-Crosier Y, de Ancos E, *et al*. Use of laser flare photometry to assess and monitor inflammation in uveitis. *Ophthalmology* 1997;104:64–72.
30 Gonzales CA, Ladas JG, Davis JL, *et al*. Relationships between laser flare photometry values and complications of uveitis. *Arch Ophthalmol* 2001;119:1763–9.
31 Davis JL, Dacanay LM, Holland GN, *et al*. Laser flare photometry and complications of chronic uveitis in children. *Am J Ophthalmol* 2003;135:763–71.
32 Tappeiner C, Heinz C, Roesel M, *et al*. Elevated laser flare values correlate with complicated course of anterior uveitis in patients with juvenile idiopathic arthritis. *Acta Ophthalmol* 2011;89:e521–e527.
33 Herbort CP, Tugal-Tutkun I. The importance of quantitative measurement methods for uveitis: laser flare photometry endorsed in Europe while neglected in Japan where the technology measuring quantitatively intraocular inflammation was developed. *Int Ophthalmol* 2017;37:469–73.
34 Heiligenhaus A, Foeldvari I, Edelsten C, *et al*. Proposed outcome measures for prospective clinical trials in juvenile idiopathic arthritis-associated uveitis: a consensus effort from the multinational interdisciplinary working group for uveitis in childhood. *Arthritis Care Res* 2012;64:1365–72.
35 Giannini EH, Ruperto N, Ravelli A, *et al*. Preliminary definition of improvement in juvenile arthritis. *Arthritis Rheum* 1997;40:1202–9.
36 Brunner HI, Lovell DJ, Finck BK, *et al*. Preliminary definition of disease flare in juvenile rheumatoid arthritis. *J Rheumatol* 2002;29:1058–64.
37 Bedrick EJ. A family of confidence intervals for the ratio of two binomial proportions. *Biometrics* 1987;43:993–7.
38 Fagerland MW, Lydersen S, Laake P. Recommended confidence intervals for two independent binomial proportions. *Stat Methods Med Res* 2015;24:224–54.
39 Becker I, Horneff G. Risk of Serious Infection in Juvenile Idiopathic Arthritis Patients Associated With Tumor Necrosis Factor Inhibitors and Disease Activity in the German Biologics in Pediatric Rheumatology Registry. *Arthritis Care Res* 2017;69:552–60.

EXTENDED REPORT

Risk of malignancy associated with paediatric use of tumour necrosis factor inhibitors

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ABSTRACT

Objective To determine whether tumour necrosis factor inhibitor (TNFi) use is associated with an increased rate of incident malignancy compared with no TNFi use in the treatment of juvenile idiopathic arthritis (JIA), paediatric inflammatory bowel disease (pIBD) and paediatric plaque psoriasis (pPsO).

Methods We performed a retrospective cohort study of administrative claims data from the USA from 2000 to 2014. Exposure to TNFi was considered permanent from the first observed exposure onward. The malignancy outcome was defined by diagnosis codes with evidence of cancer treatment. We calculated standardised incidence ratios (SIRs) comparing the observed number of malignancies to the expected numbers according to cancer surveillance data. We used multivariable Cox proportional hazards models to estimate adjusted HRs (aHRs) for incident malignancy.

Results We identified 15 598 children with TNFi use and 73 839 children with no TNFi use (30 703 and 121 801 person-years of follow-up, respectively). We identified 15 malignancies among children with TNFi use (SIR 2.9 (1.6 to 4.9)) and 42 malignancies among children without TNFi use (SIR 2.1 (1.5 to 2.9)). The aHR was 1.58 (0.88 to 2.85) for TNFi use versus no TNFi use. In pIBD, TNFi use with thiopurine use was associated with a higher SIR (6.0 (1.2 to 17.5)) compared with TNFi use without thiopurine use (2.5 (0.7 to 6.4)).

Conclusion Children diagnosed with JIA, pIBD and pPsO had an increased rate of malignancy compared with the general population, but treatment with TNFi did not appear to significantly further increase the risk compared with no TNFi use. More data are needed about the long-term risks of TNFi use.

INTRODUCTION

Tumour necrosis factor inhibitors (TNFi) are highly effective for the treatment of juvenile idiopathic arthritis (JIA)[1 2]; paediatric inflammatory bowel disease (pIBD), including Crohn's disease[3 4] and ulcerative colitis[5] ; and paediatric plaque psoriasis (pPsO).[6] Indeed, the advent of TNFi began the era of treatment with biologic agents, which has greatly improved expected outcomes in these chronic conditions.

Nevertheless, there is significant worry about the potentially increased rate of malignancy associated with paediatric use of TNFi compared with the rate in the general population, as first reported by the United States Food and Drug Administration (FDA) in 2009.[7] This initial report had many limitations, including failure to account for a possible increased risk of malignancy associated with the underlying conditions being treated with TNFi (ie, increased background risk of malignancy) or for a possible increased risk associated with other immunosuppressive medications, such as the thiopurines.[8]

To date, the very low incidence of paediatric malignancy and the relatively small number of children exposed to TNFi have limited attempts to definitively prove or disprove an independent association between paediatric TNFi use and subsequent malignancy. In this study, we used administrative claims data from the USA to determine the comparative rates of malignancy among children with JIA, pIBD and pPsO who were and were not treated with TNFi.

METHODS

Data source

After obtaining institutional review board approval, we performed this study using national US Medicaid Analytic eXtract files (MAX) (from government-provided health insurance for low-income families) from 2000 through 2010 and national US Truven MarketScan files (from commercial health insurance via employers) from 2010 through 30 September 2014. Race and ethnicity data were not available in MarketScan. We used all of the data available to us at the time of the study.

Study populations

We identified cohorts of children with the most common disease indications for TNFi. Criteria for study inclusion were (a) the first physician diagnosis code for pPsO, JIA or pIBD prior to age 18 years; and (b) at least two physician diagnosis codes for pPsO, JIA or pIBD that were >7 and <183 days apart OR any one physician diagnosis code for pPsO, JIA or pIBD that was followed by a prescription claim for immunosuppressant medications typically used to treat pPsO, JIA or pIBD (eg, methotrexate, azathioprine, TNFi) within 183 days. Patients were excluded for diagnosis codes of pPsO, JIA or IBD prior to 6 months of age and for any diagnosis codes for organ transplantation, HIV infection (HIV) or systemic lupus erythematosus (SLE) and related rheumatological conditions prior to the start of follow-up. Follow-up for each patient began 183 days after the first diagnosis code of the codes that satisfied criterion (b) above in order to exclude prevalent or misdiagnosed malignancy. Patients were excluded for any diagnosis codes for malignancy or any claims for cancer chemotherapy prior to the start of follow-up. Patients were

assigned to disease cohorts using a hierarchy (pPsO<JIA<pIBD) and could change during the course of the study (eg, a patient's diagnosis could change from JIA to pIBD, but not the reverse).

We used the same approach to identify a cohort of children diagnosed with attention-deficit hyperactivity disorder (ADHD) to evaluate the performance of our malignancy outcome algorithm, described below. In addition to the exclusions above, children were excluded from the ADHD cohort for any diagnoses of pPsO, JIA or IBD or any exposure to any of the medications of interest at any time.

Medication exposures

We identified exposure to any of the five commercially available TNFi (adalimumab, certolizumab, etanercept, golimumab, infliximab) using pharmacy and infusion claims, and all TNFi were considered together as a single exposure group. For patients with pIBD, exposure to thiopurines (azathioprine and mercaptopurine) was also assessed. For both TNFi and thiopurines, patients were considered permanently exposed from the first observed exposure onward, including exposures prior to the start of study follow-up. Exposure categories were binary, and patients could contribute follow-up time to more than one medication exposure group sequentially throughout the study according to their treatment course (eg, a patient could begin follow-up in the no TNFi use group and switch to the TNFi group on first observed exposure). We classified new TNFi users as defined by >6 months of observation without any TNFi exposure prior to the first observed TNFi exposure.

Malignancy outcome

We used an adapted version of a malignancy-finding algorithm that was previously validated in adults.[9 10] Incident malignancy was defined by at least one physician diagnosis code for malignancy accompanied by claims evidence of treatment for malignancy (ie, chemotherapy, radiation therapy or surgical excision). Diagnoses of non-melanoma skin cancer (NMSC) were ignored because they could not be sufficiently substantiated.

Study follow-up

Follow-up for all patients continued until one of the following events occurred: end of study period, malignancy outcome, loss of observability in the data (ie, loss of healthcare coverage benefits) or any diagnosis codes for organ transplantation, HIV or SLE.

Analysis

We determined the malignancy rates for the disease cohorts stratified by exposure to TNFi. We then combined results across diseases. We used Surveillance, Epidemiology, and End Results (SEER) data to calculate the expected number of malignancies according to the age, sex and race distributions of follow-up time in each cohort. We calculated standardised incidence ratios (SIRs) with 95% CIs comparing the observed number of malignancies to the expected numbers according to SEER. We separately evaluated lymphomas because of particular concern about this malignancy type.[7]

To assess the performance of the malignancy outcome algorithm, we determined the rate of malignancy in the ADHD cohort and computed age, sex and race-adjusted SIR. The ADHD cohort is expected to have a rate of malignancy comparable to the general population.

We used Cox proportional hazards models to determine the HR for incident malignancy for TNFi use compared with no TNFi use. We assessed for confounding using bivariate models including age, sex, race and data source and included variables that altered the HR for TNFi use by approximately 10% or more. Because individual patients could contribute sequentially to both cohorts, a sandwich variance estimator was applied to account for additional correlations in the data.[11] Analyses were performed using SAS V.9.4 (SAS Institute).

Table 1 Patient characteristics

Characteristic	TNFi use	No TNFi use
No of patients	15598	73839
JIA	7419 (48%)	23116 (31%)
pIBD	6808 (44%)	20049 (27%)
pPsO	1371 (9%)	30674 (42%)
Female	57%	57%
Race		
White	21%	33%
Black	7%	10%
Other/unknown	72%	57%
MAX data source (%)	41	61
Mean age in years at start of follow-up (SD)	13.4 (4.2)	11.6 (4.7)
Median age in years at start of follow-up (IQR)	14.0 (11.0–17.0)	12.0 (8.0–16.0)
Mean years of follow-up (SD)	2.0 (1.8)	1.7 (1.7)
Median years of follow-up (IQR)	1.4 (0.6–2.8)	1.1 (0.4–2.3)

5959 patients contributed to both no TNFi use and then subsequently the TNFi use cohorts.
JIA, juvenile idiopathic arthritis; MAX, Medicaid Analytic eXtract; pIBD, paediatric inflammatory bowel disease; pPsO, paediatric plaque psoriasis; TNFi, tumour necrosis factor inhibitor.

RESULTS

We identified 28005 patients with JIA, 24035 patients with pIBD and 31438 patients with PsO, including 7419/6808/1371 TNFi users, respectively (table 1). The proportion of TNFi users was much greater among JIA (26%) and pIBD (28%) compared with PsO (4%). The proportion of TNFi users was higher among patients in MarketScan (27%) compared with MAX (13%). The median duration of follow-up after first TNFi exposure was 1.4 years, and 25% of TNFi users had at least 2.8 years of follow-up after TNFi exposure. The approximate distribution of TNFi use overall was etanercept 37%, infliximab 34%, adalimumab 29%, certolizumab 2% and golimumab 1%.

Table 2 shows the incident malignancy rates and corresponding SIR compared with age, sex and race adjusted SEER estimates. The incidence of malignancies in the ADHD cohort was highly consistent with that predicted by SEER, with a resultant SIR of 0.97 (0.91–1.05).

In total, there were 15 incident malignancies identified during 30703 person-years of follow-up after TNFi use and 42 malignancies in 121801 person-years of follow-up among those without TNFi use. The 15 malignancies following TNFi use included six lymphoma, three brain, two leukaemia, two malignant melanoma, one bone and one liver. The SIRs associated with no TNFi use for JIA, pIBD and pPsO were nearly identical (2.1 (1.1–3.5), 2.1 (1.1–3.6) and 2.1 (1.1–3.5), respectively). The SIR associated with TNFi use in JIA and pIBD was very similar (3.1 (1.3–6.1) and 3.3 (1.3–6.9), respectively), and there were no malignancies identified following TNFi use among those with pPsO (SIR 0 (0–9.2)). When the results for all three indications for TNFi were combined, the SIR associated with TNFi use was 2.9 (1.6–4.9) and the SIR associated with no TNFi use was 2.1 (1.5–2.9).

Table 2 Incident malignancy rates and standardised incidence ratios

Cohort	Incident malignancies	Person-years of follow-up	Rate per 100 000 person-years	Expected malignancies according to SEER	SIR (95% CI)
JIA with TNFi use	8	16 272	49.2	2.6	3.1 (1.3 to 6.1)
JIA with no TNFi use	13	39 257	33.1	6.3	2.1 (1.1 to 3.5)
pIBD with TNFi use	7	12 189	57.4	2.1	3.3 (1.3 to 6.9)
pIBD with no TNFi use	13	35 615	36.5	6.1	2.1 (1.1 to 3.6)
pPsO with TNFi use	0	2242	(0)	0.4	0 (0 to 9.2)
pPsO with no TNFi use	16	46 929	34.1	7.5	2.1 (1.2 to 3.5)
All patients with TNFi use	15	30 703	48.9	5.1	2.9 (1.6 to 4.9)
All patients with no TNFi use	42	121 801	34.5	19.9	2.1 (1.5 to 2.9)
ADHD	745	4 663 186	16.0	765.0	0.97 (0.91 to 1.05)

ADHD, attention deficit hyperactivity disorder; SEER, Surveillance, Epidemiology, and End Results; SIR, standardised incidence ratio; TNFi, tumour necrosis factor inhibitor.

Table 3 shows the incident lymphoma rates and corresponding SIR. The incidence of lymphomas in the ADHD cohort was highly consistent with that predicted by SEER (SIR 1.05 (0.89–1.24)). In total, there were six lymphomas identified following TNFi use (three each for JIA and pIBD) and nine lymphomas among those without TNFi use (three for pIBD and six for pPsO). When all three indications for TNFi were combined, the SIR associated with TNFi use was 6.0 (2.4–14.5) and the SIR associated with no TNFi use was 2.7 (1.2–5.2).

Table 4 shows the results from Cox proportional HR models. The unadjusted HR for TNFi use versus no TNFi use was 1.45 (0.80–2.62). In bivariate models, this estimate was not confounded by age, sex or race. Adjusting for data source, the HR was 1.58 (0.88–2.85) for TNFi use versus no TNFi use. The HR for lymphoma was 2.64 (0.93–7.51) for TNFi use versus no TNFi use.

Of the 15 598 TNFi users, 9744 (62%) met the definition of new users and had 20 149 person-years (median 1.6 years) of follow-up after TNFi use. Among the TNFi new users, we observed nine incident malignancies. The median duration of time from new TNFi use to malignancy diagnosis was 343 days (range 38 to 1159), and three malignancies were diagnosed within 6 months of starting TNFi.

In the pIBD cohorts, children with thiopurine use and no TNFi use had two malignancies in 7770 person-years with an SIR of 1.5 (0.2–5.6). Children with TNFi use and no thiopurine use had four malignancies in 8913 person-years with an SIR of 2.5 (0.7–6.4). Children with TNFi use and thiopurine use had three malignancies in 2977 person-years with an SIR of 6.0 (1.2–17.5).

DISCUSSION

Despite nearly 20 years of use, there remains worry about the safety of TNFi, especially when used to treat children. This study using national administrative claims data from two sources in the USA assessed children with the three most common indications for TNFi and found no significant increased risk of overall malignancy associated with TNFi use compared with no TNFi use, although a 2-fold to 3-fold increase could not be excluded given the small number of observed malignancies. On the other hand, this study showed an approximate doubling of the incidence of malignancy associated with JIA, pIBD and pPsO in the absence of TNFi use compared with the general population. Taken together, these data suggest that, despite earlier reports to the contrary, TNFi use in childhood is not likely to substantially increase the overall risk of malignancy.

Our study confirmed previously published large studies showing an increased risk of malignancy associated with JIA in the absence of TNFi use. Large observational studies including those using linked national databases from Sweden[12] and Taiwan[13] and US claims data from MAX[14] and commercial insurers[15] have consistently found a 2-fold to 4-fold increase in malignancies in children with JIA in the absence of TNFi use compared with the general population.

There are fewer studies about the background risk of malignancy in pIBD and pPsO. Our study found an increase in incident malignancy associated with pIBD without TNFi use that was comparable with results from recently published large prospective[16] and retrospective[17] cohort studies of pIBD. Numerous previously published studies of adult IBD have shown

Table 3 Incident lymphoma rates and standardised incidence ratios

Cohort	Incident lymphomas	Person-years of follow-up	Rate per 100 000 person-years	Expected lymphomas according to SEER	SIR (95% CI)
JIA with TNFi use	3	16 272	18.4	0.4	7.5 (1.5 to 21.9)
JIA with no TNFi use	0	39 257	(0)	1.0	0 (0 to 3.7)
pIBD with TNFi use	3	12 189	24.6	0.4	7.5 (1.5 to 21.9)
pIBD with no TNFi use	3	35 615	8.4	1.1	2.7 (0.6 to 8.0)
pPsO with TNFi use	0	2242	(0)	0.08	0 (0 to 45.9)
pPsO with no TNFi use	6	46 929	12.8	1.2	5.0 (1.8 to 10.9)
All patients with TNFi use	6	30 703	19.5	0.9	6.0 (2.4 to 14.5)
All patients with no TNFi use	9	121 801	7.4	3.3	2.7 (1.2 to 5.2)
ADHD	144	4 663 186	3.1	136.9	1.05 (0.89 to 1.24)

ADHD, attention deficit hyperactivity disorder; JIA, juvenile idiopathic arthritis; pIRB, paediatric inflammatory bowel disease; pPsO, paediatric plaque psoriasis; SEER, Surveillance, Epidemiology, and End Results; SIR, standardised incidence ratio; TNFi, tumour necrosis factor inhibitor.

Table 4 HRs for the comparison of incident malignancy for TNFi use versus no TNFi use

Comparison	HR (95% CI)
TNFi use vs no TNFi use (unadjusted)	1.45 (0.80 to 2.62)
TNFi use vs no TNFi use (adjusted for data source)	1.58 (0.88 to 2.85)
MarketScan vs MAX	0.56 (0.32 to 0.99)
TNFi use vs no TNFi use, restricted to MarketScan (unadjusted)	1.13 (0.49 to 2.58)
TNFi use vs no TNFi use, restricted to MAX (unadjusted)	2.61 (1.01 to 6.79)

MAX, Medicaid Analytic eXtract; TNFi, tumour necrosis factor inhibitor.

an increased risk of several malignancies, particularly gastrointestinal cancers.[18] Our study found a similarly increased incidence of malignancy associated with pPsO without TNFi use, and several studies of adult PsO have shown an increased risk of malignancy.[19 20]

Our study adds to the growing evidence that TNFi use is likely not strongly associated with subsequent malignancy. In JIA, our preliminary prior study did not identify any incident malignancies in 2922 person-years of follow-up after TNFi use.[14] More recently, a report from the JIA German biologics registry did not observe an increase in the rate of malignancy following etanercept use compared with no TNFi use.[21] The previously mentioned large prospective cohort of patients with pIBD evaluated malignancy following any biologic use (>95% of which was TNFi use).[16] Biologic use without ever use of thiopurines was not associated with malignancy (SIR 1.11 (0.03–6.16)), while use of biologics with use of thiopurines was associated with malignancy (SIR 3.06 (1.32–6.04)). To our knowledge, there are no previously published large studies of the risk of malignancy associated with TNFi use in pPsO. In adults, large meta-analyses of clinical trials and large long-term observational studies have shown no increase in the risk of malignancy (aside from NMSC) following TNFi use in rheumatoid arthritis,[22 23] IBD[24] and PsO.[25 26]

One of the primary concerns in the initial FDA report was an increased incidence of lymphoma, up to 18 times the expected rate for children who received infliximab.[7] Our study was limited by the few number of lymphomas identified. Nevertheless, the HR for lymphoma with TNFi use was much lower than the FDA's result while still suggesting a possible true association (2.64 (0.93–7.51)). We could not adjust for high disease activity and severity, and these factors are believed to increase risk of lymphoma in adults with RA up to 70-fold compared with those with low disease activity.[27]

There are significant concerns about thiopurine use and malignancy, especially if TNFi are also used. Thiopurines alone have been shown to be associated with malignancy, particularly lymphoma, in adults with IBD.[28 29] TNFi and thiopurines together have been shown to be higher risk than TNFi alone in adults with IBD.[30] As noted above, TNFi and thiopurine use in pIBD was reported to have an approximately 2.8-fold increase in the SIR compared with TNFi use alone.[16] Our study showed a similar approximately 2.4-fold increase in the SIR for TNFi and thiopurine use compared with TNFi use alone. The small number of malignancies and the typical prescribing patterns for TNFi prevented extensive evaluation of co-medications (eg, there were few children with JIA who received TNFi without having ever received methotrexate).

The beginning and end of the potential risk window for malignancy following TNFi initiation and discontinuation are unknown. We assumed an immediate risk of infinite duration on TNFi exposure to maximise the number of malignancies attributed to TNFi. When we restricted our analyses to new users of TNFi, we observed three of nine malignancies occurred within 6 months of TNFi initiation, a time period that may be too short to be appropriately attributed to TNFi. If these three cases are excluded from the overall TNFi use cohort, then the resultant SIR is reduced to 2.4 (1.2–4.1). The median time from initiation of TNFi to development of malignancy among the cases initially reported by the FDA was approximately 2.5 years.[7] Greater than 25% of the TNFi users in this study had more than 2.5 years of observation after TNFi use, but overall long-term follow-up was limited.

A major challenge to the interpretation of studies of the association of TNFi and malignancy is confounding by indication. In studies of adults with RA[27] and IBD,[31] high disease activity and severity are directly associated with an increased rate of malignancy irrespective of treatment. Because high disease activity and severity are the very indications for paediatric use of TNFi, all observational studies are susceptible to bias towards an increased risk of malignancy associated with TNFi use. Methotrexate or thiopurines are often used prior to TNFi and may impart additional risks of malignancy. If one completely ignores the contribution of disease activity and prior treatments and attributes all of the observed increased risk of malignancy causally to TNFi use, the absolute increased rate of malignancy is still very small. We observed an absolute crude increased rate of malignancy of 14.4 per 100 000 person-years with a corresponding number needed to harm of 6944.

Our study had several important limitations. We did not have access to healthcare records to confirm the indications for TNFi or the diagnosis of malignancy. Overdiagnosis of the conditions commonly treated with TNFi among children without TNFi use would likely bias the study results against TNFi, given a background increased risk of malignancy associated with these conditions compared with the general population. Our malignancy outcome identification algorithm produced results for the ADHD cohort that were highly correlated with expected rates for overall malignancy and lymphoma. Also reassuring is the similarity between our results and the results of a prospective pIBD cohort in which pathological confirmation of malignancies was obtained.[16] It is possible that some prevalent malignancies were misclassified as incident, but the use of a 6-month period to evaluate for prevalent malignancy prior to follow-up and the accuracy of our estimates for ADHD minimised this concern. Because NMSC is not reported to SEER, we could not evaluate the accuracy of that outcome and chose not to assess its incidence in our study. Owing to the few malignancy outcomes, we were unable to assess malignancy rates associated with individual TNFi. Because the rates of malignancy among children without TNFi use were so similar among the three diseases of interest, we chose to combine all patients into a single analysis to improve the precision of the estimates, but it is possible that there is effect modification by disease. We observed numerically different HRs for malignancy in our two data sources, although this may possibly be attributable to few observed outcomes, as the estimates had overlapping 95% CI. Lastly, the amount of observable follow-up after TNFi exposure was limited, largely because of frequent changes in healthcare coverage in the USA. A possible association between long-term TNFi use and malignancy could not be adequately evaluated. Many of these challenges may be partially addressed with long-term data from large prospective observational registries.[16 21 32 33]

In conclusion, our study demonstrates that being diagnosed with JIA, pIBD or pPsO increases the risk of incident malignancy and that use of TNFi does not appear to significantly further increase this risk in the first few years after use, with the possible exception of lymphoma.

Contributors TB conceptualised and designed the study, acquired the data, interpreted the results, drafted the initial manuscript, and reviewed and revised the manuscript. FX analysed the data and critically reviewed the manuscript. LC acquired the data, analysed the data and critically reviewed the manuscript. DBH, RM and MMM interpreted the results and critically reviewed the manuscript. JDL conceptualised and designed the study, interpreted the results and critically reviewed the manuscript. KGS acquired the data, interpreted the results and critically reviewed the manuscript. JRC conceptualised and designed the study, acquired the data, interpreted the results and critically reviewed the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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REFERENCES

1 Lovell DJ, Giannini EH, Reiff A, *et al*. Etanercept in children with polyarticular juvenile rheumatoid arthritis. Pediatric Rheumatology Collaborative Study Group. *N Engl J Med* 2000;342:763–9.
2 Lovell DJ, Ruperto N, Goodman S, *et al*. Adalimumab with or without methotrexate in juvenile rheumatoid arthritis. *N Engl J Med* 2008;359:810–20.
3 Hyams J, Crandall W, Kugathasan S, *et al*. Induction and maintenance infliximab therapy for the treatment of moderate-to-severe Crohn's disease in children. *Gastroenterology* 2007;132:863–73.
4 Hyams JS, Griffiths A, Markowitz J, *et al*. Safety and efficacy of adalimumab for moderate to severe Crohn's disease in children. *Gastroenterology* 2012;143:365–74.
5 Hyams J, Damaraju L, Blank M, *et al*. Induction and maintenance therapy with infliximab for children with moderate to severe ulcerative colitis. *Clin Gastroenterol Hepatol* 2012;10:391–9.
6 Paller AS, Siegfried EC, Langley RG, *et al*. Etanercept treatment for children and adolescents with plaque psoriasis. *N Engl J Med* 2008;358:241–51.
7 Diak P, Siegel J, La Grenade L, *et al*. Tumor necrosis factor alpha blockers and malignancy in children: forty-eight cases reported to the Food and Drug Administration. *Arthritis Rheum* 2010;62:2517–24.
8 Cron RQ, Beukelman T. Guilt by association—what is the true risk of malignancy in children treated with etanercept for JIA? *Pediatr Rheumatol Online J* 2010;8:23.
9 Setoguchi S, Solomon DH, Glynn RJ, *et al*. Agreement of diagnosis and its date for hematologic malignancies and solid tumors between medicare claims and cancer registry data. *Cancer Causes Control* 2007;18:561–9.
10 Haynes K, Beukelman T, Curtis JR, *et al*. Tumor necrosis factor α inhibitor therapy and cancer risk in chronic immune-mediated diseases. *Arthritis Rheum* 2013;65:48–58.
11 Lin DY, Wei LJ. The robust inference for the Cox proportional hazards model. *J Am Stat Assoc* 1989;84:1074–8.
12 Simard JF, Neovius M, Hagelberg S, *et al*. Juvenile idiopathic arthritis and risk of cancer: a nationwide cohort study. *Arthritis Rheum* 2010;62:3776–82.
13 Kok VC, Horng JT, Huang JL, *et al*. Population-based cohort study on the risk of malignancy in East Asian children with juvenile idiopathic arthritis. *BMC Cancer* 2014;14:634.
14 Beukelman T, Haynes K, Curtis JR, *et al*. Rates of malignancy associated with juvenile idiopathic arthritis and its treatment. *Arthritis Rheum* 2012;64:1263–71.
15 Nordstrom BL, Mines D, Gu Y, *et al*. Risk of malignancy in children with juvenile idiopathic arthritis not treated with biologic agents. *Arthritis Care Res* 2012;64:1357–64.
16 Hyams JS, Dubinsky MC, Baldassano RN, *et al*. Infliximab is not associated with increased risk of malignancy or hemophagocytic lymphohistiocytosis in pediatric patients with inflammatory bowel disease. *Gastroenterology* 2017;152:1901–14.
17 Olén O, Askling J, Sachs MC, *et al*. Childhood onset inflammatory bowel disease and risk of cancer: a Swedish nationwide cohort study 1964–2014. *BMJ* 2017;358:j3951.
18 Beaugerie L, Itzkowitz SH. Cancers complicating inflammatory bowel disease. *N Engl J Med* 2015;372:1441–52.
19 Pouplard C, Brenaut E, Horreau C, *et al*. Risk of cancer in psoriasis: a systematic review and meta-analysis of epidemiological studies. *J Eur Acad Dermatol Venereol* 2013;27(Suppl 3):36–46.
20 Kimball AB, Schenfeld J, Accortt NA, *et al*. Cohort study of malignancies and hospitalized infectious events in treated and untreated patients with psoriasis and a general population in the United States. *Br J Dermatol* 2015;173:1183–90.
21 Horneff G, Klein A, Oommen PT, *et al*. Update on malignancies in children with juvenile idiopathic arthritis in the German BIKER Registry. *Clin Exp Rheumatol* 2016;34:1113–20.
22 Mariette X, Matucci-Cerinic M, Pavelka K, *et al*. Malignancies associated with tumour necrosis factor inhibitors in registries and prospective observational studies: a systematic review and meta-analysis. *Ann Rheum Dis* 2011;70:1895–904.
23 Lopez-Olivo MA, Tayar JH, Martinez-Lopez JA, *et al*. Risk of malignancies in patients with rheumatoid arthritis treated with biologic therapy: a meta-analysis. *JAMA* 2012;308:898–908.
24 Nyboe Andersen N, Pasternak B, Basit S, *et al*. Association between tumor necrosis factor-α antagonists and risk of cancer in patients with inflammatory bowel disease. *JAMA* 2014;311:2406–13.
25 Dommasch ED, Abuabara K, Shin DB, *et al*. The risk of infection and malignancy with tumor necrosis factor antagonists in adults with psoriatic disease: a systematic review and meta-analysis of randomized controlled trials. *J Am Acad Dermatol* 2011;64:1035–50.
26 Asgari MM, Ray GT, Geier JL, *et al*. Malignancy rates in a large cohort of patients with systemically treated psoriasis in a managed care population. *J Am Acad Dermatol* 2017;76:632–8.
27 Baecklund E, Iliadou A, Askling J, *et al*. Association of chronic inflammation, not its treatment, with increased lymphoma risk in rheumatoid arthritis. *Arthritis Rheum* 2006;54:692–701.
28 Kotlyar DS, Lewis JD, Beaugerie L, *et al*. Risk of lymphoma in patients with inflammatory bowel disease treated with azathioprine and 6-mercaptopurine: a meta-analysis. *Clin Gastroenterol Hepatol* 2015;13:847–58.
29 Pasternak B, Svanström H, Schmiegelow K, *et al*. Use of azathioprine and the risk of cancer in inflammatory bowel disease. *Am J Epidemiol* 2013;177:1296–305.
30 Osterman MT, Sandborn WJ, Colombel JF, *et al*. Increased risk of malignancy with adalimumab combination therapy, compared with monotherapy, for Crohn's disease. *Gastroenterology* 2014;146:941–9.
31 Beaugerie L, Svrcek M, Seksik P, *et al*. Risk of colorectal high-grade dysplasia and cancer in a prospective observational cohort of patients with inflammatory bowel disease. *Gastroenterology* 2013;145:166–75.
32 Beukelman T, Kimura Y, Ilowite NT, *et al*. The new Childhood Arthritis and Rheumatology Research Alliance (CARRA) registry: design, rationale, and characteristics of patients enrolled in the first 12 months. *Pediatr Rheumatol Online J* 2017;15:30.
33 Beukelman T, Anink J, Berntson L, *et al*. A survey of national and multi-national registries and cohort studies in juvenile idiopathic arthritis: challenges and opportunities. *Pediatr Rheumatol Online J* 2017;15:31.

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EXTENDED REPORT

Determinants of psychological well-being in axial spondyloarthritis: an analysis based on linked claims and patient-reported survey data

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ABSTRACT

Objectives The aim of this study was to assess the psychological well-being and to analyse factors associated with depressive symptoms in axial spondyloarthritis (axSpA).

Methods A stratified random sample of subjects with a diagnosis of axSpA (International Classification of Diseases, Tenth Revision, German Modification M45) was drawn from health insurance data in Germany. These persons received a postal questionnaire on disease-related, psychological and lifestyle factors as well as socioeconomic status. Additional information to verify the axSpA diagnosis was also collected. The psychological well-being was assessed by means of the 5-item WHO Well-Being Index (WHO-5), which is considered a screening tool for depression. The following established cut-offs on the WHO-5 were applied: >50: good well-being, no depressive symptoms; 29–50: mild depressive symptoms; ≤28: moderate-to-severe depressive symptoms. Information on comorbidities, drug prescriptions and non-pharmacological treatment was retrieved from claims data and linked to the questionnaire data.

Results A total of 1736 persons with a confirmed axSpA diagnosis were included. Using the cut-offs on the WHO-5, 533 persons (31%) were found to have moderate-to-severe depressive symptoms, 479 (28%) had mild depressive symptoms and 724 (42%) had a good well-being. Multivariable logistic regression revealed that higher disease activity, higher level of functional impairment, lower income, self-reported stress and lack of exercise, and younger age represent factors associated with moderate-to-severe depressive symptoms.

Conclusions The prevalence of depressive symptoms in axSpA subjects is high and associated with disease-related parameters, socioeconomic status and lifestyle factors. These findings highlight the need for the careful evaluation of depressive symptoms as a part of the management strategy for axSpA.

INTRODUCTION

Axial spondyloarthritis (axSpA) is a chronic inflammatory disease characterised by predominant involvement of the spine and/or sacroiliac joints. AxSpA comprises non-radiographic axSpA (nr-axSpA, without definite radiographic sacroiliitis) and radiographic axSpA (also known as ankylosing spondylitis (AS), characterised by the presence of radiographic sacroiliitis according to the modified New York criteria).[1] The leading symptom of axSpA is chronic back pain with onset in early adulthood, usually before age 45. In addition to back pain, peripheral articular (arthritis, enthesitis, daktylitis) and extra-articular manifestations (EAMs), such as uveitis, psoriasis and inflammatory bowel disease (IBD), contribute to the total burden of axSpA.[2]

Psychological distress, including depressive symptoms, is frequently reported in persons with axSpA.[3 4] Furthermore, a recent study showed that AS subjects have an increased risk of developing depressive disorders following their diagnosis.[5]

The objective of this study was to assess the psychological well-being and to identify factors associated with depressive symptoms in a large nationwide group of persons with axSpA by taking advantage of the linkage of claims data and self-reported patient outcomes from a survey within the Linking Patient-Reported Outcomes with CLAIms data for health services research in Rheumatology network.[6]

METHODS

Patients and study design

Data for this study were obtained from a nationwide statutory health insurance fund (BARMER) with 6.6 million members aged 18–79 years in 2014 who were continuously insured in 2013 and 2014. Among those, 21 892 had an outpatient claim with an axSpA diagnosis (International Classification of Diseases, Tenth Revision, German Modification (ICD-10-GM) code M45) in at least two quarters of the year 2014. Out of the 21 892 axSpA subjects, a stratified random sample of 5000 persons (500 within each stratum) was drawn, with stratification based on age group (18–39, 40–49, 50–59, 60–69 and 70–79 years) and sex. The sample size was determined so that mean effect sizes of 0.25 could be detected with a power of 80%, even if subgroups from certain age/sex strata were compared. A questionnaire was sent out in autumn 2015, gathering information on rheumatological care ('Are you currently being treated by a rheumatologist?'), confirmation of axSpA diagnosis ('How is the disease called by your physician?'), disease-related, psychological and lifestyle factors, as well as socioeconomic status. Persons who had not answered within 4 weeks received a reminder.

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Claims data

Age, sex, EAMs (including uveitis, psoriasis and IBD), comorbidities and pharmacological and non-pharmacological treatment were retrieved from claims data from 2015. Comorbidities and EAMs were identified via ICD-10-GM codes and drug prescriptions via the anatomical therapeutic chemical classification, where at least one outpatient claim had to be documented. Non-steroidal anti-inflammatory drugs (NSAIDs), opioids, non-opioid analgesics, biological disease-modifying antirheumatic drugs (bDMARDs), glucocorticoids and conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) comprised axSpA-related treatment. Non-pharmacological treatment was represented by physiotherapy, including manual therapy, exercise therapy and therapist massages.

Questionnaire data

The psychological well-being/presence of depressive symptoms was assessed using the 5-item WHO Well-Being Index (WHO-5). It is a short, generic global index based on five positively phrased items measuring the subjective psychological well-being of the respondents over the past 2 weeks.[7] The five items are: (1) 'I have felt cheerful and in good spirits', (2) 'I have felt calm and relaxed', (3) 'I have felt active and vigorous', (4) 'I woke up feeling fresh and rested' and (5) 'My daily life has been filled with things that interest me'. They are scored by using 6-point Likert scales (0–5) for each item.[8] The total of the five scales generates the 0–25 WHO-5 raw score, with higher scores indicating better well-being. The raw score is translated to the 0–100 WHO-5 (percentage) score by multiplying by 4. A cut-off score of ≤28 on the WHO-5 was used to denote the possible presence of moderate-to-severe depressive symptoms. Scores of 29–50 on the WHO-5 indicate mild depressive symptoms, whereas scores of >50 suggest good well-being/no depressive symptoms. The screening performance of the WHO-5 has been validated in previous studies.[8–10] A cut-off score of ≤28 on the WHO-5 was tested against the Structured Clinical Interview for the Diagnostic and Statistical Manual (DSM)-IV as the criterion standard for the presence of 'major depressive disorder', with a sensitivity of 94% and a specificity of 78%.[11]

To validate the diagnosis of axSpA obtained via claims data, persons were asked to confirm the presence of the diagnosis of axSpA/AS. Further, persons were asked about the occurrence (ever) of EAMs. Information about the diagnosing and treating physician, age of symptom onset, age of diagnosis, HLA-B27 status, disease activity and functional status were also collected via questionnaire. The activity of axSpA was assessed using the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)[12] and the functional status by means of the Bath Ankylosing Spondylitis Functional Index (BASFI).[13] Socioeconomic status was determined using household income and type of work arrangement. Lifestyle factors comprised the characteristics body mass index (BMI), lack of exercise, smoking tobacco and perception of suffering from stress.

Statistical analysis

The total number of persons returning the questionnaires who gave their consent for linking questionnaire data to claims data was weighted according to the sex and age group distribution of the source population. Weighted subgroup analyses were performed on those who confirmed their axSpA diagnosis. Descriptive statistics (mean, SE of the mean (SEM) and percentages) were used to describe differences between the groups of persons screened as having no, mild or moderate-to-severe depressive symptoms. The SEM was used instead of SD due to the stratified nature of the study sample. Significant differences were assessed using one-way analyses of variance for continuous variables and using Rao-Scott χ^2 tests otherwise. Tests resulting in p values <0.05 were considered statistically significant.

Stepwise multivariable logistic regression analysis was used to determine factors associated with moderate-to-severe depressive symptoms in persons with axSpA, adjusting for the main demographic (age and sex), disease-related (information on rheumatological care, HLA-B27 status, disease activity and functional status, presence of IBD, uveitis and psoriasis, pharmacological treatment with NSAIDs, opioids, non-opioid analgesics, bDMARDs, csDMARDs and glucocorticoids, non-pharmacological treatment with physiotherapy), lifestyle (BMI, lack of exercise, smoking tobacco and perception of suffering from stress) and socioeconomic (household income, full-time employment) characteristics. A significance level of 0.03 was required to allow a variable into the model, and a significance level of 0.05 was required for a variable to stay in the model. Age and sex were always included in the model. Adjusted ORs were calculated with a 95% CI.

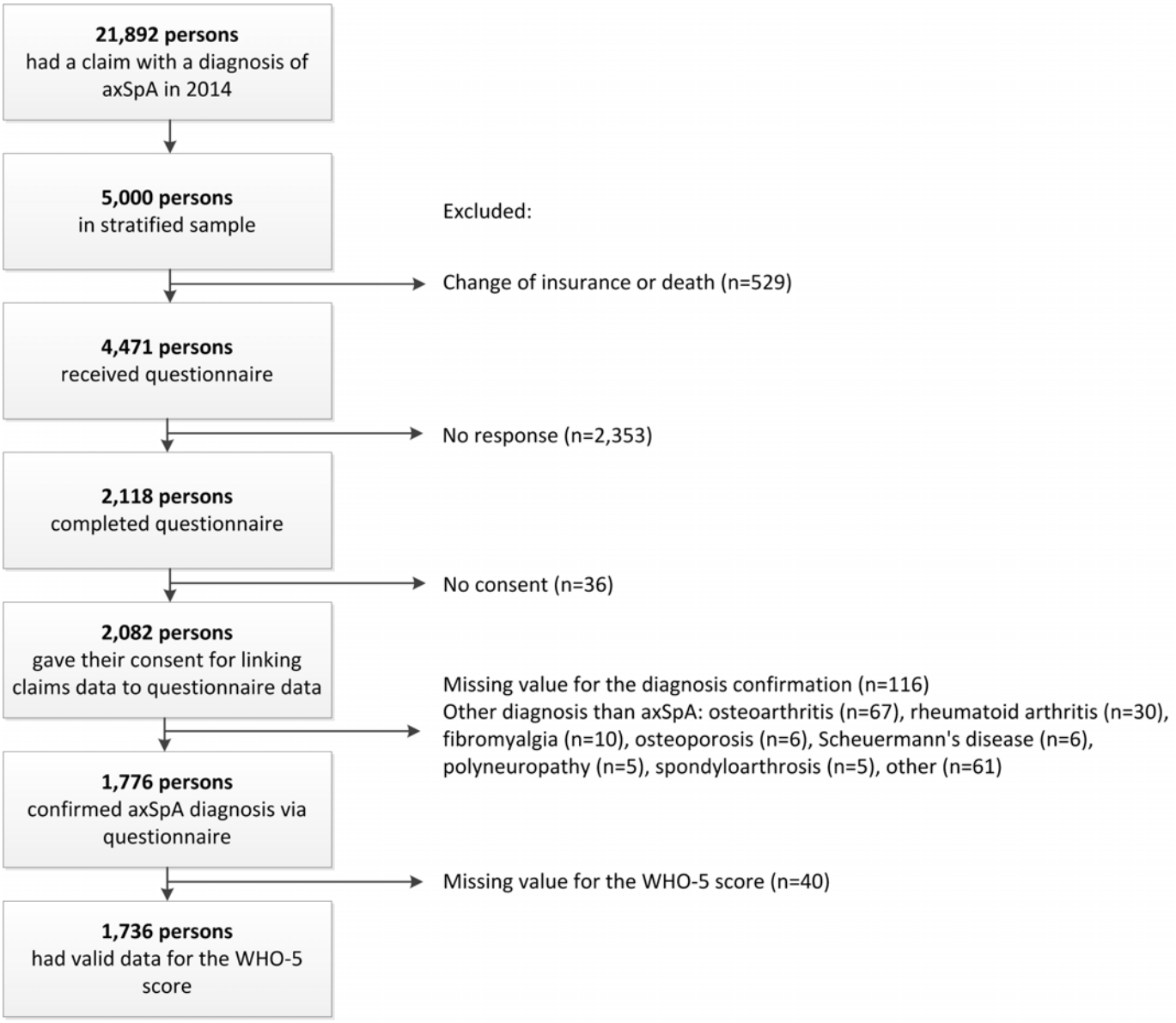
Data analyses were performed with SAS V.9.4 using procedures for complex survey designs (SURVEYMEANS, SURVEYFREQ and SURVEYLOGISTIC), which incorporated the stratified design into the analyses.

RESULTS

A total of 4471 persons (original sample of 5000 persons minus those who had changed their insurance or died) received the questionnaire (figure 1). Of those, a total of 2118 persons responded (47%) and 2082 gave their consent for linking questionnaire data to claims data of whom 1776 persons confirmed their axSpA diagnosis via questionnaire (85%). The remaining 15% reported diagnoses other than axSpA and were excluded from the analysis, including 5.6% who did not report their diagnosis. A total of 1736 persons had valid data for the WHO-5 score and were therefore included in the analysis. The main demographic, disease-related, lifestyle and socioeconomic characteristics are presented in table 1. All variables obtained from questionnaire data had a maximum of 4% of missing values, except for the variables household income (6% missing values) and HLA-B27 status (31% missing values).

Among the 1736 persons with confirmed axSpA, 724 (42%) had a WHO-5 score of >50, suggesting good well-being, 479 (28%) had a WHO-5 score of 29–50, indicating mild depressive symptoms, and 533 (31%) had a WHO-5 score ≤28, denoting the possible presence of moderate-to-severe depressive symptoms. Table 1 also gives an overview of the patients' characteristics in each of the three groups according to the WHO-5. Persons considered as having a good well-being were more often men and aged ≥60 than persons screened as having mild or moderate-to-severe depressive symptoms. Persons with a low score on the WHO-5 were more often provided with rheumatological care compared with persons with a medium or high score on the WHO-5. Statistically significant differences between the three WHO-5 groups were observed in disease activity and functional status: BASDAI and BASFI scores were poorest among persons with moderate-to-severe depressive symptoms and best in persons with good well-being.

The prevalence/self reported occurrence of psoriasis and IBD was higher in persons with moderate-to-severe depressive symptoms as compared with persons with good well-being or mild depressive symptoms, even though differences in the prevalence



AxSpA, Axial Spondyloarthritis; WHO-5, 5-item WHO Well-Being Index.

Figure 1 Flow chart of the study population.

of psoriasis according to the claims data did not reach the level of statistical significance (table 1). At the same time, the prevalence/self reported occurrence of uveitis was similar across the subgroups.

Statistically significant differences between the three WHO-5 groups were also observed in household income, full-time employment, self-reported lack of exercise, perception of suffering from stress, tobacco smoking and BMI. Persons with moderate-to-severe depressive symptoms less often had a high household income and full-time employment than persons with good well-being. More than half of persons with a low WHO-5 score reported a perception of suffering from stress compared with one-fourth of persons with a high WHO-5 score. Self-reported lack of exercise and tobacco smoking were also more often reported among persons with a low WHO-5 score than among persons with a high WHO-5. BMI scores were higher among persons with moderate-to-severe depressive symptoms compared with persons with good well-being.

No statistically significant differences between persons with a low, medium or high WHO-5 score were found with respect to treatment with bDMARDs and csDMARDS. However, persons with moderate-to-severe depressive symptoms more often received NSAIDs, analgesics and glucocorticoids compared with persons considered as having a good well-being. Furthermore, significant differences between the WHO-5 groups were found in treatment with proton pump inhibitors (table 3). However, in persons with no NSAIDs use, the differences between the WHO-5 groups in treatment with proton pump inhibitors were no longer significant. More patients with moderate-to-severe depressive symptoms received pharmacological treatment for SpA in general compared with patients with good well-being. Physiotherapy was more often prescribed for persons with a low WHO-5 score than for persons with a medium or high WHO-5 score.

Most frequent comorbidities (prevalence of ≥10% in at least one WHO-5 group) and their treatments are shown in table 2

Table 1 Main demographic, disease-related, lifestyle and socioeconomic characteristics of patients with axSpA

	Total	Depressive symptoms			
	n=1736	No n=724 (42%)	Mild n=479 (28%)	Moderate/severe n=533 (31%)	P value
Sex, female	46.3	41.0	50.6	49.7	**0.0008**
Age, years	55.8±0.1	57.4±0.4	54.1±0.5	55.1±0.4	**<0.0001**
Symptom duration	25.2±0.3	26.6±0.5	23.9±0.6	24.3±0.6	**0.0013**
Duration since diagnosis	19.4±0.3	21.3±0.5	17.9±0.6	18±0.6	**<0.0001**
In rheumatological care	46.1	39.5	47.2	54.0	**<0.0001**
HLA-B27 positive	86.0	87.4	83.5	86.6	0.2816
BASDAI, 0–10	4.5±0	3.3±0.1	4.8±0.1	5.8±0.1	**<0.0001**
BASFI, 0–10	4.1±0.1	2.9±0.1	4.2±0.1	5.6±0.1	**<0.0001**
IBD (claims data)	5.5	4.9	3.9	7.8	**0.0164**
IBD (ever, self-reported)	8.8	6.9	6.2	13.9	**<0.0001**
Uveitis (claims data)	13.7	14.4	14.9	11.8	0.2969
Uveitis (ever, self-reported)	27.3	28.7	28	24.9	0.3181
Psoriasis (claims data)	9.5	8.3	9.7	11.1	0.2789
Psoriasis (ever, self-reported)	15.1	12.1	16.8	17.7	**0.0152**
Body mass index, kg/m²	27±0.1	26.8±0.2	27±0.2	27.4±0.2	0.0872
Lack of exercise	24.4	19.0	25.3	30.8	**<0.0001**
Suffering from stress	39.9	25.5	47.8	52.6	**<0.0001**
Full-time employment	31.7	32.2	35.6	27.4	**0.0187**
Household income, €					
<1500	25.9	20.1	26.7	33.1	**< 0.0001**
1500–3200	56.0	55.0	58.3	55.2	
>3200	18.1	24.9	15.0	11.7	
Smoking, current	18.9	14.8	20.0	23.4	**0.0006**
Pharmacological treatment					
NSAIDs	59.7	50.9	63.4	68.6	**<0.0001**
Non-opioid analgesics	22.6	18.2	20.6	30.3	**<0.0001**
Opioids	16	9.4	16.5	24.4	**<0.0001**
bDMARDs*	17.1	15.5	19.1	17.5	0.2520
csDMARDs†	11.7	10.6	10.5	14.4	0.0818
Glucocorticoids	18.3	15.6	17.5	22.8	**0.0046**
No pharmacological treatment	22.1	30.3	21.2	11.8	**<0.0001**
Physiotherapy	49.7	45.6	48.1	56.6	**0.0006**

Values are presented as mean±SE of the mean for continuous characteristics and as percentages otherwise. P values were assessed using analyses of variance for continuous characteristics and Rao-Scott χ^2 tests otherwise.
P values <0.05 are shown in bold.
*bDMARDs: 17.0% tumour necrosis factor blocker, 0.07% secukinumab, 0.06% tocilizumab, 0.06% ustekinumab, 0.05% abatacept.
†csDMARDs: 5.7% sulfasalazine, 5.6% methotrexate, 0.9% leflunomide, 0.6% azathioprine, 0.2% ciclosporin.
AxSpA, axial spondyloarthritis; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; bDMARDs, biological disease-modifying antirheumatic drugs; csDMARDs, conventional synthetic disease-modifying antirheumatic drugs; IBD, inflammatory bowel disease; NSAIDs, non-steroidal anti-inflammatory drugs.

and table 3, respectively. Remarkably, the prevalence of mental and neurological disorders was higher in patients with depressive symptoms according to WHO-5 compared with patients with good well-being. They also most frequently received antidepressants, anxiolytics, hypnotics and sedatives. In addition, the prevalence of fibromyalgia increased with increasing level of depressive symptoms.

Univariable logistic regression models showed that BASDAI, BASFI, sex, household income, perception of suffering from stress and self-reported lack of exercise were associated with a low WHO-5 score, whereas age was not associated (table 4). Stepwise multivariable logistic regression analysis revealed that higher BASDAI and BASFI, perception of suffering from stress, self-reported lack of exercise, as well as lower income level and younger age were factors associated with moderate-to-severe depressive symptoms while controlling for the other variables (table 4). Here, we additionally entered sex in the final model since it is a biologically meaningful parameter but was not selected by the stepwise procedure. However, sex was not associated with moderate-to-severe depressive symptoms while controlling for the other variables (table 4).

DISCUSSION

The objective of this nationwide population-based study was to assess the psychological well-being and its associated factors in axSpA subjects to raise the awareness of such factors on the patient level and to manage adequate axSpA therapy on the healthcare level. In a number of studies evaluated in a recent review, the WHO-5 demonstrated an adequate validity. This review showed that the WHO-5 is a highly useful tool that can be applied in clinical practice.[14]

Table 2 The most frequent comorbidities* according to the claims data from 2015 in patients with axSpA

	Total	Depressive symptoms			
	n=1736	No n=724 (42%)	Mild n=479 (28%)	Moderate/severe n=533 (31%)	P value
Cardiovascular diseases					
Hypertensive diseases (I10–I15)	51.5	51.5	49.1	53.6	0.3624
Ischaemic heart diseases (I20–I25)	12.5	13.4	11.1	12.4	0.5153
Diseases of arteries (I70–I79)	9.5	9	8.5	11.1	0.3208
Diseases of veins (I80–I89)	18.4	17.3	17.3	21	0.2059
Mental disorders					
Depressive disorders (F32, F33)	22.2	12.6	22.9	34.6	**<0.0001**
Anxiety disorders (F40, F41)	9.5	6.1	8.4	14.9	**<0.0001**
Reaction to severe stress, and adjustment disorders (F43)	9.8	7.3	7.6	15.3	**<0.0001**
Somatoform disorders (F45)	21.2	14.7	21.2	29.9	**<0.0001**
Neurological disorders					
Nerve, nerve root and plexus disorders (G50–G59)	11.7	9	9.8	17.1	**<0.0001**
Polyneuropathies (G60–G64)	8.4	5.9	7.9	12.2	**0.0006**
Sleep disorders (G47)	9.9	8.5	9.6	12.2	0.1022
Obstructive sleep apnoea (G47.31)	2.9	2.5	3.7	2.7	0.4671
Musculoskeletal disorders (other than axSpA)					
Osteoarthritis (M15–M19)	35.9	34.6	36.2	37.5	0.5771
Spondylosis (M47)	24.2	18.8	24.3	31.4	**<0.0001**
Other soft tissue disorders, not elsewhere classified (M79)	26.4	22.6	24.2	33.5	**<0.0001**
Fibromyalgia (M79.7)	4.5	1.9	4.9	7.7	**<0.0001**
Disorders of bone density (M80–M85)	13.1	14	13.1	12	0.5929
Metabolic and endocrine disorders					
Disorders of thyroid gland (E00–E07)	28.2	28	28.2	28.6	0.9775
Diabetes mellitus (E10–E14)	16.2	16.9	14.2	16.9	0.4036
Type two diabetes mellitus (E11)	14.3	14.2	12.9	15.6	0.5172
Overweight (E65–E68)	14.7	14.4	12.6	17.2	0.1295
Respiratory tract diseases					
Chronic obstructive pulmonary disease (J44)	8.8	9.3	6.3	10.2	0.0790
Asthma bronchiale (J45)	9.9	9.2	9.2	11.5	0.3269
Gastrointestinal diseases					
Diseases of oesophagus, stomach and duodenum (K20–K31)	24.5	23.4	23.4	27	0.2874

Values are presented as percentages. P values were assessed using Rao-Scott χ^2 tests. P values <0.05 are shown in bold.
*With prevalence of ≥10% in at least one WHO-5 group excluding axSpA and EAMs.
AxSpA, axial spondyloarthritis; EAMs, extra-articular manifestations.

Using a cut-off score of ≤28 on the WHO-5, we found that 31% of persons with axSpA had moderate-to-severe depressive symptoms; with the cut-off score of ≤50, an additional 28% with mild depressive symptoms would be added to the previous number, yielding a total of 59% of patients with depressive symptoms/impaired well-being. This is consistent with the results of previous studies. For example, a study conducted by Barlow *et al*[15] reported that about one-third of AS subjects presented a high level of depressive symptoms according to the Centre for Epidemiological Studies-Depression (CES-D) scale.[16] A national study in Sweden showed that the consultation rate for depression was increased by >60% in AS patients compared with the background population seeking care.[17] In a nationwide population-based study of psychiatric disorders among patients with AS in Taiwan, an increased risk of depressive, anxiety and sleep disorders in AS subjects was found compared with general populations.[5] We found a mean WHO-5 score of 44.70 in axSpA subjects, which is considerably below the WHO-5 score of 69.95 reported among the population in Germany aged 41–60 years.[18] However, the prevalence of depressive symptoms according to the WHO-5 among axSpA subjects is similar to 54% among German patients aged 50–64 years with rheumatoid arthritis reported in a recent study.[19] For comparison, Busch *et al*[20] assessed current depressive symptoms with the 9-item Patient Health Questionnaire among the adult population in Germany and reported a prevalence of depressive symptoms of 8.1%.

We found a clear and statistically significant association between patient-reported depressive symptoms derived from the WHO-5 score and both physician-reported mental disorders and the use of antidepressants according to the claims data, confirming the validity of the results. The same was also true for anxiety, adjustment and somatoform disorders (and drugs used for the treatment of mental disorders), as well as fibromyalgia—their prevalence was significantly higher in patients with higher level of depressive symptoms.

Furthermore, we found statistically significant differences in the prevalence/self reported occurrence of IBD among the WHO-5 groups which is consistent with a current study focused on IBD and depression.[21] The same applies to psoriasis which is known to be associated with depression, as well as fibromyalgia.[22]

In general, persons with more depressive symptoms tended to have more frequently also other comorbidities not directly related to SpA as indicated in table 2. This indicates that the presence of other chronic disease other than SpA and related EAMs might

Table 3 Treatment of comorbidities according to the claims data from 2015 in patients with axSpA

	Total	Depressive symptoms			
	n=1736	No n=724 (42%)	Mild n=479 (28%)	Moderate/severe n=533 (31%)	P value
Cardiovascular diseases					
Antihypertensive agents (C02, C07, C08, C09)	51	51.1	49.3	52.4	0.6261
Antithrombotic agents (B01A)	15.2	15.1	13.8	16.5	0.5283
Diuretics (C03)	13.3	11.9	14.2	14.5	0.3464
Mental and neurological disorders					
Antidepressants (N06A)	16.9	9.9	18.5	24.9	**<0.0001**
Antiepileptic drugs (N03)	6.4	3.9	5.2	10.7	**<0.0001**
Psycholeptic drugs (N05)	6.2*	4.5	4.6	10	**<0.0001**
Metabolic and endocrine disorders					
Thyroid hormones (H03AA)	19.4	18.3	19.6	20.8	0.5638
Lipid modifying agents (C10)	18.1	19.2	17.9	16.6	0.5117
Insulins and analogues (A10A)	3.8	4.4	4.2	2.6	0.2384
Blood glucose-lowering drugs, excluding insulins (A10B)	8.3	9.3	5.1	10	**0.0152**
Respiratory tract diseases					
Drugs for obstructive airway diseases (R03)	14.4	13.4	11.4	18.4	**0.0050**
Gastrointestinal diseases					
Proton pump inhibitors (A02BC)	42.3	35.9	44.4	49.3	**<0.0001**

Values are presented as percentages. P values were assessed using Rao-Scott χ^2 tests. P values <0.05 are shown in bold.
*Psycholeptic drugs: 1.8 % antipsychotics, 3.1 % anxiolytics, 2.5 % hypnotics and sedatives.
AxSpA, axial spondyloarthritis.

significantly affect well-being. There were statistically significant differences between the WHO-5 groups in the frequency of administrations of oral antidiabetic drugs and drugs for obstructive pulmonary disease (table 3) with the highest use in persons with moderate-to severe depressive symptoms. Given no significant differences in the prevalence of the corresponding diagnoses, this data might indicate a higher severity of diabetes and obstructive pulmonary disease in persons with the worst depressive symptoms.

Previous studies showed that patients with axSpA with depressive symptoms have increased disease activity,[23 24] impaired functional status[25] and work disability.[26–29] In our study, we found that higher disease activity, functional limitations, perception of suffering from stress, self-reported lack of exercise and lower income and younger age were factors associated with the risk of moderate-to-severe depressive symptoms in persons with axSpA while controlling for the other variables.

What is the practical meaning of these findings? First, the practical relevance is related to a high prevalence of depressive symptoms indicating that a substantial proportion of persons with axSpA might suffer from depression requiring intervention that is not recognised by treating physicians. Such an impaired subjective well-being might affect the perception of pain and other axSpA-related symptoms and therefore on the patient-reported outcomes relevant for the therapy. Indeed, in our study, patients with depressive symptoms had higher BASDAI and BASFI scores and more frequently received NSAIDs and analgesics (including opioids) in comparison with the patients considered as having a good psychological well-being. However, higher disease activity and a higher level of functional disability (as indicated by BASDAI and BASFI) might be indicators of a severe disease resulting in the development of depressive symptoms and requiring more intensive therapy. In this case, the reduction of disease activity would also improve psychological well-being.

The same is true for the relationship between behavioural and socioeconomic factors (lack of exercise, perception of stress and low income)—they may be a cause but in some cases also a consequence of depression. However, if the causal role of these factors is true, at least some of them (lack of exercise and perception of stress) are potentially modifiable and should therefore be considered in the patients' management.

Table 4 Factors associated with the presence of symptoms suggestive of depression (WHO-5 score of ≤28): results from univariable and multivariable logistic regression analyses

		OR (95% CI)	
	Reference	Univariable analysis	Multivariable analysis
Sex, female	Male	**1.22 (1.00 to 1.48)**	1.00 (0.77 to 1.29)
Age	Per 10 years	1.00 (0.99 to 1.00)	**0.98 (0.97 to 0.99)**
BASDAI	Per unit	**1.65 (1.56 to 1.75)**	**1.37 (1.27 to 1.49)**
BASFI	Per unit	**1.38 (1.33 to 1.44)**	**1.25 (1.17 to 1.33)**
Lack of exercise	No	**1.62 (1.30 to 2.03)**	**1.50 (1.14 to 1.98)**
Suffering from stress	No	**2.12 (1.73 to 2.60)**	**2.03 (1.55 to 2.64)**
Household income, <€ 1500	>€ 3200	**2.62 (1.88 to 3.66)**	**1.88 (1.27 to 2.78)**
Household income, € 1500–3200	>€ 3200	**1.77 (1.30 to 2.40)**	**1.54 (1.08 to 2.19)**

Odds ratios of variables associated with a WHO-5 score of ≤ 28 are shown in bold .
BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; WHO-5, 5-item WHO Well-Being Index.

The high prevalence of depressive symptoms that are potentially not recognised by physicians is also clinically relevant in the context of new drugs currently under investigation for the treatment of axSpA, which might worsen depressive symptoms and/or provoke suicidal behaviour like apremilast, a phosphodiesterase-4 inhibitor,[30] or brodalumab, a monoclonal antibody against interleukin-17 receptor.[31]

We also found an interesting negative association of age with the presence of depressive symptoms. This might indicate that with increasing age, patients with axSpA are able to cope with the disease better, despite increasing non-SpA-related comorbidities (that showed no significant association with depressive symptoms in our analysis), leading to a lower prevalence of depressive symptoms.

Our study has strengths and limitations. The main strength of the present study was the linkage of a large nationwide claims database to questionnaire data in patients with axSpA. Claims data represent a very valid source of data on drug prescriptions, healthcare utilisation and comorbidities, while questionnaire data contained valuable additional information on disease-related, psychological, socioeconomic and lifestyle factors normally not available via claims data. The linkage of the questionnaire data to the claims data allowed for the validation of key variables, such as the diagnosis and the presence of depressive symptoms.

The primary limitation of the present study was its cross-sectional design, which did not allow us to determine the direction of significant associations or to investigate the consequences of depressive symptoms on the long-term outcome of axSpA. A prospective cohort or interventional study design is required to answer the question of a causal relationship. However, such a relationship between depressive symptoms and its associated factors may act in both directions with a substantial individual variation in the strength and direction of the association. Furthermore, claims data are normally collected for administrative rather than for scientific purposes, and the recorded diagnoses must be interpreted with caution. However, we validated the initial diagnosis from the claims data against the self-reported diagnosis obtained from the questionnaire and selected only patients who confirmed the presence of axSpA; as a result, the characteristics of the resulting group in terms of age, sex distribution, prevalence of EAMs and therapy are comparable to those of prospectively recruited axSpA cohorts.[32–34] Finally, we used only a simple screening tool (WHO-5) to assess patients' psychological well-being/depressive symptoms. The WHO-5 has been validated in several studies (usually in non-rheumatological indications) as a sensitive and specific tool for the detection of depression. Nonetheless, the specific validation of the tool with the confirmation of the presence/absence of depression by a specialist has not yet been performed for patients with axSpA.

Conclusion

In summary, we found a high prevalence of depressive symptoms/impaired psychological well-being in patients with axSpA. Higher BASDAI and BASFI, the perception of suffering from stress, lack of exercise, lower income level and younger age are factors associated with moderate-to-severe depressive symptoms in patients with axSpA while controlling for other variables. These findings highlight the need for the careful evaluation of depressive symptoms as a part of the management strategy for axSpA, helping to improve axSpA outcomes.

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Competing interests None declared.

Patient consent Detail has been removed from this case description/these case descriptions to ensure anonymity. The editors and reviewers have seen the detailed information available and are satisfied that the information backs up the case the authors are making.

Ethics approval The study was approved by the ethics committee of the Charité - Universitätsmedizin Berlin, Berlin, Germany.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Rudwaleit M, van der Heijde D, Landewé R, *et al*. The development of Assessment of SpondyloArthritis international Society classification criteria for axial spondyloarthritis (part II): validation and final selection. *Ann Rheum Dis* 2009;68:777–83.
2 Bremander A, Petersson IF, Bergman S, *et al*. Population-based estimates of common comorbidities and cardiovascular disease in ankylosing spondylitis. *Arthritis Care Res* 2011;63:550–6.
3 Zou Q, Jiang Y, Mu F, *et al*. Correlation of Axial Spondyloarthritis with Anxiety and Depression. *Med Sci Monit* 2016;22:3202–8.
4 Kilic G, Kilic E, Ozgocmen S. Relationship between psychiatric status, self-reported outcome measures, and clinical parameters in axial spondyloarthritis. *Medicine* 2014;93:e337.
5 Shen CC, Hu LY, Yang AC, *et al*. Risk of Psychiatric Disorders following Ankylosing Spondylitis: A Nationwide Population-based Retrospective Cohort Study. *J Rheumatol* 2016;43:625–31.
6 Hense S, Luque Ramos A, Callhoff J, *et al*. Prävalenz der rheumatoiden Arthritis in Deutschland auf Basis von Kassendaten: Regionale Unterschiede und erste Ergebnisse der PROCLAIR-Studie. [Prevalence of rheumatoid arthritis in Germany based on health insurance data: Regional differences and first results of the PROCLAIR study]. *Z Rheumatol* 2016;75:819–27.
7 Bech P. Health-related quality of life measurements in the assessment of pain clinic results. *Acta Anaesthesiol Scand* 1999;43:893–6.
8 Bonsignore M, Barkow K, Jessen F, *et al*. Validity of the five-item WHO Well-Being Index (WHO-5) in an elderly population. *Eur Arch Psychiatry Clin Neurosci* 2001;251(Suppl 2):II27–31.
9 Inagaki H, Ito K, Sakuma N, *et al*. [Reliability and validity of the simplified Japanese version of the WHO-Five Well-being Index (S-WHO-5-J)]. *Nihon Koshu Eisei Zasshi* 2013;60:294–301.
10 Hajos TR, Pouwer F, Skovlund SE, *et al*. Psychometric and screening properties of the WHO-5 well-being index in adult outpatients with Type 1 or Type 2 diabetes mellitus. *Diabet Med* 2013;30:e63–9.
11 Löwe B, Spitzer RL, Gräfe K, *et al*. Comparative validity of three screening questionnaires for DSM-IV depressive disorders and physicians' diagnoses. *J Affect Disord* 2004;78:131–40.
12 Garrett S, Jenkinson T, Kennedy LG, *et al*. A new approach to defining disease status in ankylosing spondylitis: the Bath Ankylosing Spondylitis Disease Activity Index. *J Rheumatol* 1994;21:2286–91.
13 Calin A, Garrett S, Whitelock H, *et al*. A new approach to defining functional ability in ankylosing spondylitis: the development of the Bath Ankylosing Spondylitis Functional Index. *J Rheumatol* 1994;21:2281–5.
14 Topp CW, Østergaard SD, Søndergaard S, *et al*. The WHO-5 Well-Being Index: a systematic review of the literature. *Psychother Psychosom* 2015;84:167–76.
15 Barlow JH, Macey SJ, Struthers GR. Gender, depression, and ankylosing spondylitis. *Arthritis Care Res* 1993;6:45–51.
16 Radloff LS. The CES-D Scale. *Appl Psychol Meas* 1977;1:385–401.
17 Meesters JJ, Bremander A, Bergman S, *et al*. The risk for depression in patients with ankylosing spondylitis: a population-based cohort study. *Arthritis Res Ther* 2014;16:418.

18 Brähler E, Mühlan H, Albani C, *et al*. Teststatistische Prüfung und Normierung der deutschen Versionen des EUROHIS-QOL Lebensqualität-Index und des WHO-5 Wohlbefindens-Index. *Diagnostica* 2007;53:83–96.
19 Jobski K, Luque Ramos A, Albrecht K, *et al*. Pain, depressive symptoms and medication in German patients with rheumatoid arthritis-results from the linking patient-reported outcomes with claims data for health services research in rheumatology (PROCLAIR) study. *Pharmacoepidemiol Drug Saf* 2017;26:766–74.
20 Busch MA, Maske UE, Ryl L, *et al*. [Prevalence of depressive symptoms and diagnosed depression among adults in Germany: results of the German Health Interview and Examination Survey for Adults (DEGS1)]. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz* 2013;56(5-6):733–9.
21 Kochar B, Barnes EL, Long MD, *et al*. Depression Is Associated With More Aggressive Inflammatory Bowel Disease. *Am J Gastroenterol* 2018;113:80–5.
22 Chang MH, Hsu JW, Huang KL, *et al*. Bidirectional Association Between Depression and Fibromyalgia Syndrome: A Nationwide Longitudinal Study. *J Pain* 2015;16:895–902.
23 Baysal O, Durmuş B, Ersoy Y, *et al*. Relationship between psychological status and disease activity and quality of life in ankylosing spondylitis. *Rheumatol Int* 2011;31:795–800.
24 Brionez TF, Assassi S, Reveille JD, *et al*. Psychological correlates of self-reported disease activity in ankylosing spondylitis. *J Rheumatol* 2010;37:829–34.
25 Brionez TF, Assassi S, Reveille JD, *et al*. Psychological correlates of self-reported functional limitation in patients with ankylosing spondylitis. *Arthritis Res Ther* 2009;11:R182.
26 Verstappen SM, Watson KD, Lunt M, *et al*. Working status in patients with rheumatoid arthritis, ankylosing spondylitis and psoriatic arthritis: results from the British Society for Rheumatology Biologics Register. *Rheumatology* 2010;49:1570–7.
27 Barlow JH, Wright CC, Williams B, *et al*. Work disability among people with ankylosing spondylitis. *Arthritis Rheum* 2001;45:424–9.
28 Ward MM, Kuzis S. Risk factors for work disability in patients with ankylosing spondylitis. *J Rheumatol* 2001;28:315–21.
29 Marengo MF, Schneeberger EE, Citera G, *et al*. Work status among patients with ankylosing spondylitis in Argentina. *J Clin Rheumatol* 2008;14:273–7.
30 Crowley J, Thaçi D, Joly P, *et al*. Long-term safety and tolerability of apremilast in patients with psoriasis: Pooled safety analysis for ≥156 weeks from 2 phase 3, randomized, controlled trials (ESTEEM 1 and 2). *J Am Acad Dermatol* 2017;77:310–7.
31 Danesh MJ, Kimball AB. Brodalumab and suicidal ideation in the context of a recent economic crisis in the United States. *J Am Acad Dermatol* 2016;74:190–2.
32 Rudwaleit M, Haibel H, Baraliakos X, *et al*. The early disease stage in axial spondylarthritis: results from the German Spondyloarthritis Inception Cohort. *Arthritis Rheum* 2009;60:717–27.
33 Ciurea A, Scherer A, Exer P, *et al*. Tumor necrosis factor α inhibition in radiographic and nonradiographic axial spondyloarthritis: results from a large observational cohort. *Arthritis Rheum* 2013;65:3096–106.
34 Kiltz U, Baraliakos X, Karakostas P, *et al*. Do patients with non-radiographic axial spondylarthritis differ from patients with ankylosing spondylitis? *Arthritis Care Res* 2012;64:1415–22.

EXTENDED REPORT

Sialendoscopy enhances salivary gland function in Sjögren's syndrome: a 6-month follow-up, randomised and controlled, single blind study

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ABSTRACT

Objectives To assess the effect of sialendoscopy of the major salivary glands on salivary flow and xerostomia in patients with Sjögren's syndrome (SS).

Methods Forty-nine patients with SS were randomly assigned to a control group (n=15) and two intervention groups: irrigation of the major glands with saline (n=16) or with saline followed by triamcinolone acetonide (TA) in saline (n=18). Unstimulated whole saliva flow (UWS), chewing-stimulated whole saliva flow (SWS), citric acid-stimulated parotid flow (SPF), Clinical Oral Dryness Score (CODS), Xerostomia Inventory (XI) score and the European League Against Rheumatism (EULAR) SS Patient-Reported Index (ESSPRI) were obtained 1 week (T0) before, and 1 (T1), 8 (T8), 16 (T16) and 24 (T24) weeks after sialendoscopy.

Results Median baseline UWS, SWS and SPF scores were 0.14, 0.46 and 0.22 mL/min, respectively. After intervention, significant increases in UWS and SWS were observed in the saline group (at T8 (P=0.013) and T24 (P=0.004)) and the saline/TA group (at T24 (P=0.03) and T=16 (P=0.035)). SPF was increased significantly in the saline/TA group at T24 (P=0.03). XI scores declined after sialendoscopy in both intervention groups. Compared with the control group, CODS, XI and ESSPRI improved in the intervention groups. UWS, SWS and SPF were higher in the intervention groups compared with the control group, but these differences were not significant except for SPF in the saline/TA group at T24 (P=0.005).

Conclusions Irrigation of the major salivary glands in patients with SS enhances salivary flow and reduces xerostomia up to 6 months after sialendoscopy.

INTRODUCTION

Sjögren's syndrome (SS) is an autoimmune disorder causing chronic inflammation and irreversible damage of the exocrine glands. SS is characterised by mononuclear infiltrates and IgG plasma cells in salivary and lacrimal glands which lead to irreversible destruction of glandular tissue.[1] SS affects 0.01%–4% of the population, with a female-to-male ratio of 9:1.[2–5] SS causes a gradual reduction in the quantity and quality of saliva.[6] Because of hyposalivation, patients with SS suffer from a sensation of oral dryness (xerostomia) and its related complaints (eating and swallowing problems, lack of taste, speech problems), and are prone to develop progressive dental decay and inflammation of the oral mucosa.[7]

No effective treatment is available for SS or its related hyposalivation. Systemic treatment is often ineffective and can result in major side effects.[8] However, some biologic disease-modifying antirheumatic drugs have shown promise for improved efficacy with mostly mild adverse events.[9] Biologicals will probably not be effective for all patients with SS, but only in subgroups of patients with SS.[10]

No effective therapy is currently available that reduces complications associated with SS.[11]

In a recent case series and in two pilot studies, sialendoscopy of the major salivary glands appeared to alleviate symptoms of SS and improve salivary function.[12–14] Sialendoscopy is used for diagnostic purposes as well as to treat chronic obstructive salivary disorders caused by strictures, mucus plugs and sialoliths (figure 1).[15–19] Irrigation of the ductal system, either with saline or a solution of saline and corticosteroids, was suggested to alleviate complaints in patients affected by salivary gland inflammatory diseases and xerostomia.[12–14 20]

The aim of this study was to assess the effect of sialendoscopy with saline or saline followed by saline/corticosteroids on salivary gland function, oral dryness and symptoms in patients with SS. Comparisons were made between these treatments, baseline levels and non-treatment controls.

METHODS

Study population

Patients with SS between 18 and 75 years of age with a baseline unstimulated whole saliva flow (UWS) >0.0 mL/min or evidence of glandular reserve function (stimulated baseline whole saliva flow (SWS) ≥0.02 mL/min) were included. All patients fulfilled the 2002 American–European Consensus Group classification criteria.[21]

Patients with acute sialadenitis, severe illness, physical conditions interfering with a treatment under general anaesthesia or a history of head and neck radiotherapy were excluded. Use of sialogogues was not allowed during the study. Written consent was obtained from each patient.

Study design

Participants were randomly assigned to a non-intervention control group (n=15) or two sialendoscopy (intervention) groups: irrigation of the ductal system with saline (n=16) or with saline followed by triamcinolone acetonide 40 mg/mL (TA; Kenacort-A 40; Bristol-Myers Squibb, New

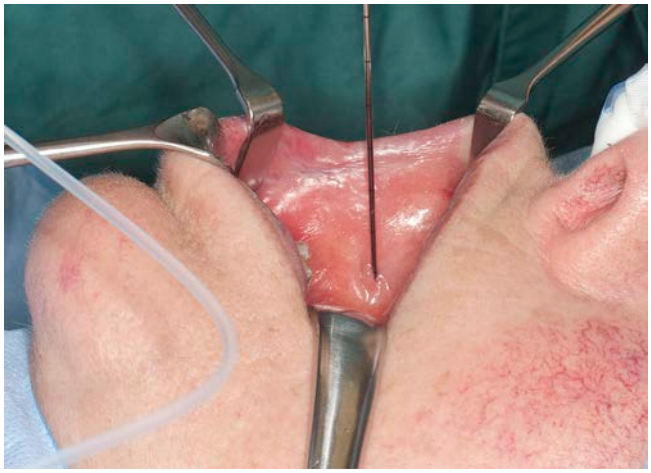

Figure 1 During the procedure, a sialendoscope is introduced into the orifice of the parotid duct after dilation of the papilla.

York, USA) in 5 mL saline at the end of the procedure (n=18). Controls were not blinded on their allocation to the non-intervention group. Such a design would have required sham sialendoscopy in controls, which was not permitted by the Research Ethics Board.

In all groups, UWS, SWS and stimulated parotid flow (SPF) were collected and measured at five research appointments: 1 week before intervention (T0), and 1 (T1), 8 (T8), 16 (T16) and 24 (T24) weeks after intervention. The Clinical Oral Dryness Scale (CODS),[22] Xerostomia Inventory (XI) score[23] and the European League Against Rheumatism (EULAR) SS Patient-Reported Index (ESSPRI)[24] were scored at every appointment. The study protocol is registered at the US National Institutes of Health (ClinicalTrials.gov; number: NCT02112019). The design and reporting of this study agrees with the Consolidated Standards of Reporting Trials statement.[25]

Randomisation

Participants were randomly assigned (blocked randomisation), using randomising software (www.randomizer.org), to either the non-intervention control group or an intervention group.

Outcome measures

Sialometry

Patients were instructed to refrain from eating/chewing, drinking, brushing teeth and smoking for 90 min prior to each visit. To minimise diurnal variation, all appointments were planned on the same time of the day and in the same room (temperature 21°C±2°C, humidity 50%–60%). To collect UWS, patients were instructed to start collecting saliva immediately after an initial swallow, and subsequently expectorate into a preweighed container every 30 s for a 5 min period. To collect SWS, patients were asked to chew a 5×5 cm sheet of paraffin (Parafilm M, Pechiney, Chicago, USA) and expectorate into a preweighed container every 30 s during a 5 min period. Reweighing each container after collection and subtracting the weight of the empty container determined UWS and SWS flow rates. Values are expressed as millilitres per minute.[26] Parotid-stimulated saliva was collected in plastic tubes from each parotid gland using modified Lashley cups. Stimulation was with citric acid (2% w/v) applied with a cotton wool swab to the lateral border of the tongue at 30 s intervals.[27] All assessments were performed by the same observer (FM), blinded for the therapeutic interventions (saline vs saline/TA) and condition of the patients.

Clinical Oral Dryness Score

The CODS is a validated clinical guide designed to assess oral dryness by clinical and visual inspection of the oral cavity based on several signs of oral dryness such as presence of frothy saliva and stickiness of the dental mirror to the tongue or buccal fold.[22 28] The scores for each of the 10 features were added together, resulting in score from 0 (no oral dryness) to 10 (extreme oral dryness).

Xerostomia Inventory

The summated XI is a validated questionnaire containing 11 questions about mouth feel and oral dryness, using a five-point Likert scale to indicate the frequency of symptoms. Scores from the 11 questions are added together, resulting in a total XI score varying from 11 (no dry mouth) to 55 (extremely dry mouth).[23]

EULAR SS Patient-Reported Index

ESSPRI is a patient-administered questionnaire to assess disease symptoms on a 10-point scale for pain, fatigue and dryness. ESSPRI is sensitive for measuring changes in disease symptoms after therapeutic intervention. Only the dryness domain was used in the analysis. A change of two or more points was considered clinically relevant.[24]

Intervention

Sialendoscopy was performed by one experienced surgeon (KHK). Sialendoscopy consisted of irrigation of the ductal system of both parotid and submandibular glands with saline or with saline followed by 40 mg/mL TA in 5 mL saline at the end of the procedure. Saline/TA was injected intraductally under direct vision and maintained in the glands by temporarily occluding the ductal orifices with a microvascular clamp until the end of general anaesthesia (±10 min). Strictures were dilated using hydrostatic pressure. Sialoendoscopy was performed using 0.8 or 1.1 mm diameter Erlangen sialendoscopes (Karl Storz GmbH & Co, Tuttlingen, Germany). Sialendoscopy was performed under general anaesthesia in order to standardise treatment among patients and to avoid patient discomfort because of the operation time (45 min).

Sample size and statistical analysis

A sample size of 14 patients per group was calculated, based on a previously performed pilot study, using PS power software.[13 29] Differences between time points within the three groups were examined using Wilcoxon signed-rank tests (data without normal distribution) or analysis of variance for repeated measurements (normally distributed data). Assumption of sphericity was tested with Mauchly's test. Differences between groups were assessed using the Mann-Whitney U test (data without normal distribution) or independent t-test (normally distributed data). The assumption of homogeneity of variances was tested via Levene's F-test. If the assumption of homogeneity of variances was rejected, the Welch-Satterthwaite method was used to adjust the degrees of freedom. Data were analysed with SPSS V.22.0 (IBM, Armonk, USA). A P value of 0.05 or lower was considered statistically significant.

RESULTS

Between July 2014 and November 2016, 51 patients were included. The last patient ended the follow-up period in May

Table 1 Characteristics of the study population and baseline values for all parameters

	Mean±SD or n (%)	Median (IQR)
Patient variables		
Age (years)	59 (10.37)	59.7 (54–67.1)
Female gender, n (%)	43 (87.8%)	
Disease duration (years)*	10±8.9	7 (3–13)
Control group	10.4±8.7	7.5 (3–18.8)
Saline group	8.1±9.7	6.5 (3–7.5)
Saline/TA group	11.1±8.8	11 (2.5–16.5)
Primary SS, n (%)†	34 (68%)	
Control group	9 (60%)	
Saline group	13 (81.25%)	
Saline/TA group	12 (66.7%)	
Secondary SS, n (%)†	15 (30%)	
Control group	6 (40%)	
Saline group	3 (18.75%)	
Saline/TA group	6 (33.3%)	
Autoantibodies to anti-SSA or anti-SSB§	43 (87.8%)	
Positive salivary gland biopsy	39 (79.6%)	
Objective ocular involvement (Schirmer test)	47 (96%)	
Baseline UWS (mL/min)	0.14±0.15	0.1 (0.0–0.19)
Control group	0.13±0.11	0.09 (0.03–0.18)
Saline group	0.17±0.21	0.1 (0.04–0.19)
Saline/TA group	0.13±0.11	0.1 (0.06 – 0.17)
Baseline SWS (mL/min)	0.46±0.44	0.3 (0.13–0.7)
Control group	0.49±0.46	0.25 (0.15–0.73)
Saline group	0.43±0.21	0.25 (0.07–0.7)
Saline/TA group	0.46±0.40	0.37 (0.13–0.62)
Baseline SPF (mL/min)	0.22±0.26	0.17 (0.05–0.37)
Control group	0.20±0.21	0.17 (0.00–0.47)
Saline group	0.18±0.24	0.11 (0.00–0.21)
Saline/TA group	0.28±0.30	0.18 (0.03–0.47)
XI	44.6±6.3	46 (41–50)
ESSPRI (all domains)‡	6.7±1.64	
ESSPRI (dryness domain)	7.6±1.52	
Clinical Oral Dryness Score	2.74±1.15	2 (2–3.5)
Gland variables		
Total number of glands accessible and rinsed	100 (73.5%)	
Glands accessible and rinsed—saline group	48 (75%)	
Parotid glands	30 (93.8%)	
Submandibular glands	18 (56.3%)	
Glands accessible and rinsed—saline/TA group	52 (72.2%)	
Parotid glands	34 (94.4%)	
Submandibular glands	14 (38.9%)	

Both mean (±SD) and median (IQR) are presented for non-normally distributed data. The number of glands successfully rinsed during sialendoscopy is presented.
*Disease duration is defined as years since diagnosis.
†Classified according to the 2002 American–European Consensus Group Criteria. All patients classified as secondary SS suffered from rheumatoid arthritis.§anti-SSA, anti-SSB.
‡Defined as the total ESSPRI score divided by 3.
anti-SSA, anti Sjögren's Syndrome related antigen A; anti-SSB, anti Sjögren's Syndrome related antigen B; ESSPRI, EULAR Sjögren's Syndrome Patient-Reported Index; SPF, stimulated parotid flow; SS, Sjögren's syndrome; SWS, stimulated whole saliva flow; TA, triamcinolone acetonide; UWS, unstimulated whole saliva flow; XI, Xerostomia Inventory.

2017. Characteristics of the study population are given in table 1 and allocation to the various groups is shown in figure 2.

The overall rate of complications was limited, and the complications were minor. A complicating factor was that it was not possible to identify or dilate the papilla to introduce the sialendoscope in all salivary glands. Analysis of the data for normality revealed that ESSPRI was normally distributed, and UWS, SWS, SPF, CODS and XI were not (Shapiro-Wilk; $P<0.001$). During sialendoscopy, strictures were present and removed for all treated salivary glands. Baseline comparison of the groups revealed no significant difference in outcome measures. Median UWS, SWS, SPF, CODS, XI and mean ESSPRI (dryness domain) scores are presented in tables 2 and 3 and figure 3. The percentage of patients in whom any improvement in salivation was observed after 24 weeks was 87.5% for UWS and 75% for SWS in the saline group and 72.2% for UWS and 61.1% for SWS in the saline/TA group. The percentage of patients who regained an adequate salivary flow (defined as UWS >0.1 mL/min and SWS >0.5 mL/min) after 24 weeks was 68.8% for UWS and 37.5% for SWS in the saline group and 66.7% for UWS and 55.6% for SWS in the saline/TA group. In the control group, measures did not change significantly in comparison to baseline.

Within group analysis: saline group

In the saline group, UWS increased after intervention and significant differences were found at T8 (median (Mdn)=0.14 mL/min; $Z=-2.49$, $P=0.013$, $r=-0.62$) and T16 (Mdn=0.13 mL/min; $Z=-2.35$, $P=0.019$, $r=-0.59$) compared with T0 (Mdn=0.1 mL/min).

SWS increased after intervention and significant differences were found at T24 (Mdn=0.30 mL/min; $Z=-2.90$, $P=0.004$, $r=-0.73$) compared with T0 (Mdn = 0.25 mL/min). A comparable effect was found for CODS. CODS decreased after intervention and a statistically significant difference was found at T1 (Mdn=1.5; $Z=-2.40$, $P=0.016$, $r=-0.6$) compared with T0 (Mdn=3).

XI scores in the saline group were lower after intervention at all time points compared with baseline. XI was significantly lower at T16 (Mdn=42; $Z=-2.22$, $P=0.027$, $r=-0.56$) and T24 (Mdn=38; $Z=-2.36$, $P=0.018$, $r=-0.59$) compared with T0 (Mdn=45), suggesting that sialendoscopy resulted in a reduced dry mouth feeling 16 and 24 weeks after intervention. Although numerically lower after intervention, no significant change in ESSPRI score was found in the saline group.

Within groups analysis: saline/TA group

In the saline/TA group, UWS increased after intervention and a significant difference was found at T24 (Mdn=0.12 mL/min; $Z=-2.18$, $P=0.03$, $r=-0.51$) compared with T0 (Mdn=0.1 mL/min). Furthermore, significant differences for UWS were found between T24 and T1 ($P=0.03$) and T8 ($P=0.007$).

SWS increased after intervention and a significant difference was found at T=16 (Mdn=0.64 mL/min; $Z=-2.11$, $P=0.035$, $r=-0.50$) compared with T0 (Mdn=0.37 mL/min). In this group, SPF increased over time and a significant difference was found between T0 (Mdn=0.18 mL/min) and T24 (Mdn=0.34 mL/min; $Z=-2.16$, $P=0.03$, $r=-0.51$).

A comparable effect was found for the CODS. CODS decreased after intervention and a statistically significant difference was found between T0 (Mdn=2) and T1 (Mdn=1.5; $Z=-3.09$, $P=0.002$, $r=-0.73$). All subsequent time points were significantly different compared with T0 indicating a more moist oral mucosa.

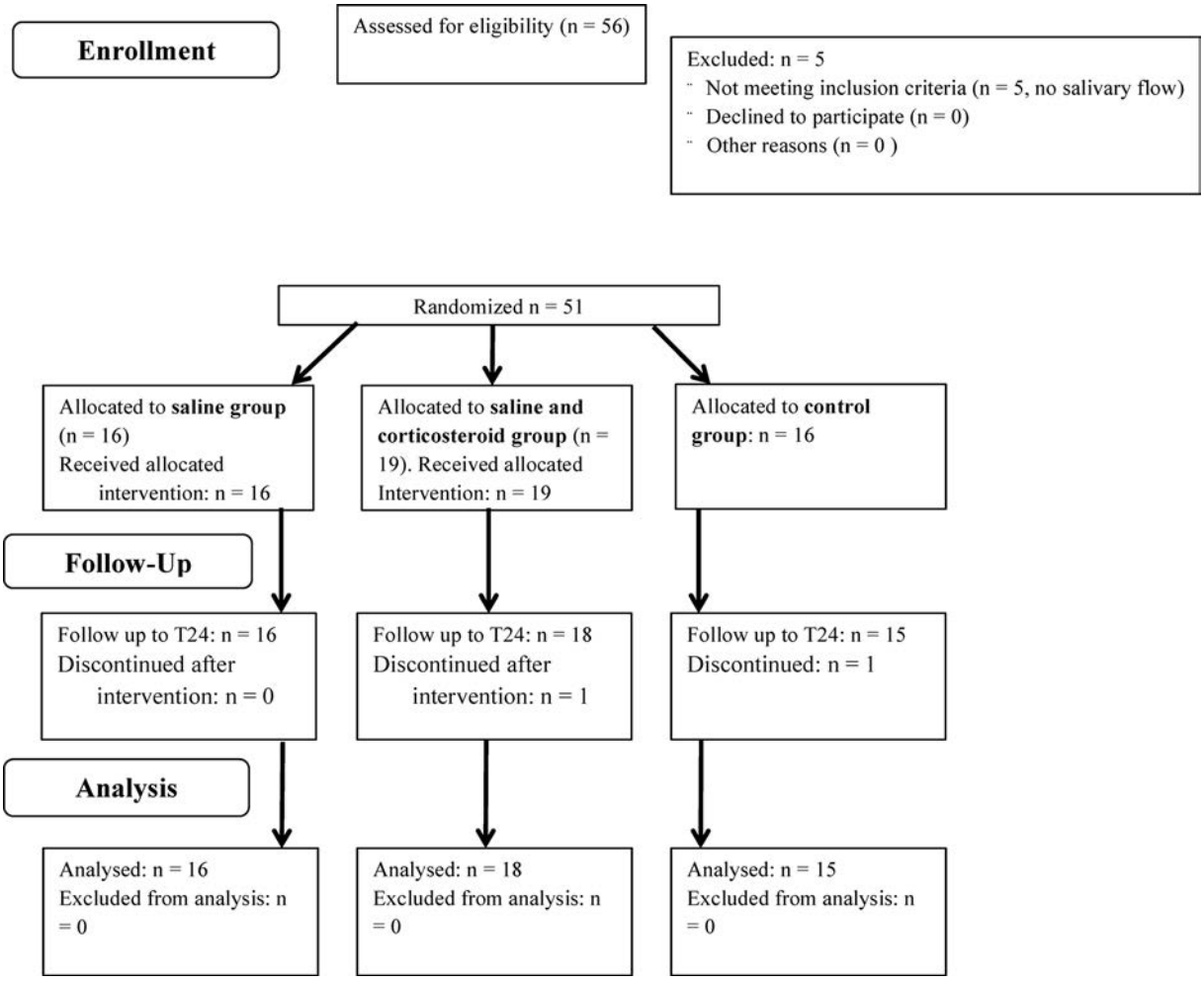

Figure 2 Flow diagram showing the allocation of participants to the various treatment groups. T24, 24 weeks after intervention.

XI scores in the saline/TA group were lower after intervention at all time points compared with baseline. XI was significantly lower at T8 (Mdn=44; Z=−2.17, P=0.03, r=−0.51) and T16 (Mdn=42.5; Z=−2.31, P=0.021, r=−0.54) compared with T0 (Mdn=45.5), suggesting that sialendoscopy resulted in a reduced dry mouth feeling 8 and 16 weeks after intervention. ESSPRI was significantly lower between T0 and T8 (P<0.001; 95% CI 0.87 to 2.51), T16 (P=0.006; 95% CI 0.51 to 2.55) and T24 (P=0.017; 95% CI 0.28 to 2.55). There was no violation of the assumption of sphericity: $\chi^2(9)=10.66$, P=0.30.

Between group analysis: saline group versus control group

When comparing the saline group with the control group, no significant difference was found for UWS, SWS and SPF at any time point. A significant difference was found for CODS at T1 between the control group (Mdn=2) and the saline intervention group (Mdn=1.5; U=69, P=0.038, r=−0.49). XI scores in the saline group were significantly lower at T1 (Mdn=42; U=62, P=0.02, r=−0.54), T16 (Mdn=42; U=57, P=0.013, r=−0.59) and T24 (Mdn=38; U=45.5, P=0.003, r=−0.70) compared with the corresponding time points in the control group (table 2) indicating a reduction in xerostomia up to 6 months, after sialendoscopy with saline. Reduction of xerostomia was also found in the dryness domain of ESSPRI. ESSPRI scores were significantly (P<0.05) lower in the saline group compared with the control group at all time points after intervention. Levene's F-test showed that there was a significant difference in the variances between the groups at T24. Therefore, the Welch-Satterthwaite method was used to adjust the degrees of freedom. This had no effect on the results for T24.

Between group analysis: saline/TA group versus control group

When comparing the saline/TA group with the control group at the different time points, no significant difference was found for salivary flow at any time point except for SPF at T24, which was significantly higher in the saline/TA group (U=58, P=0.005, r=−0.40). CODS was significantly (P<0.05) lower in the saline/TA intervention group compared with the control group at all time points (table 1) indicating a more moist oral cavity.

XI scores in the saline/TA group were lower at all time points after intervention compared with the control group (table 1). But these differences were not significant.

For the ESSPRI, significant score differences were found between the saline/TA and control group at T8 (t(31)=3.49, P=0.01; 95% CI 0.83 to 3.13), T16 (t(31)=3.77, P=0.01; 95% CI 0.99 to 3.30) and T24 (t(31)=2.16, P=0.03; 95% CI 0.87 to 3.05). Levene's F-test showed that there was a significant difference in the variances between the groups at T24. Therefore, the Welch-Satterthwaite method was used to adjust the degrees of freedom. This had no effect on the results for T24.

Table 2 Median and IQR for UWS, SWS, SPF, CODS and XI scores for all groups and time points

	Control group		Saline group		Saline/TA group	
	Median	IQR	Median	IQR	Median	IQR
UWS (mL/min)						
T0	0.09	0.03–0.18	0.10 (*, †)	0.04–0.19	0.10 (‡)	0.06–0.17
T1	0.08	0.04–0.21	0.10	0.03–0.50	0.11 (§)	0.05–0.22
T8	0.07	0.04–0.27	0.14 (*)	0.07–0.48	0.09 (¶)	0.06–0.22
T16	0.10	0.02–0.28	0.13 (†)	0.04–0.45	0.11	0.05–0.27
T24	0.12	0.03–0.22	0.16	0.07–0.38	0.12 (‡, §, ¶)	0.08–0.27
SWS (mL/min)						
T0	0.25	0.15–0.73	0.25 (*)	0.07–0.70	0.37 (‡)	0.13–0.62
T1	0.18	0.11–0.74	0.35	0.08–0.72	0.36 (§, ¶, †)	0.20–0.60
T8	0.22	0.16–0.71	0.33	0.08–0.67	0.45 (§, **, ††)	0.18–0.77
T16	0.24	0.10–0.56	0.33	0.09–0.68	0.64 (‡, ¶, **)	0.17–0.90
T24	0.25	0.11–0.67	0.30 (*)	0.09–0.81	0.61 (†, ††)	0.19–0.80
SPF (mL/min)						
T0	0.17	0.00–0.47	0.11	0.00–0.22	0.18 (*)	0.03–0.47
T1	0.05	0.00–0.65	0.03	0.00–0.21	0.11 (†)	0.03–0.35
T8	0.13	0.00–0.41	0.08	0.01–0.30	0.16	0.06–0.41
T16	0.06	0.00–0.66	0.09	0.00–0.33	0.22	0.05–0.42
T24	0.06 (¶)	0.00–0.26	0.02 (§)	0.00–0.50	0.34 (*, †, ¶, §)	0.19–0.73
CODS (1–10)						
T0	3	2–4	3 (*)	2–4	2 (‡, ††, §§, §)	2–3
T1	3 (¶)	2–3	1.5 (*, ¶)	0–3	1.5 (‡)	1–2
T8	2	2–4	1.5	1–3.75	2 (††, ‡‡)	0.75–3
T16	2	1–4	2	1–3	1 (§§)	0–2
T24	2	2–3	1.5	1–3	1 (§, ‡‡)	0–2
XI (11–55)						
T0	48	41–51	45 (*, †)	41.25–48.75	45.5 (‡, §)	38.5–50
T1	48	41–51	42	34.50–46	44.5	39–50.25
T8	47	40–50	41	34–46	44 (‡)	37.50–47.25
T16	46	42–52	42 (*)	32.25–45.75	42.5 (§)	31.50–48.50
T24	47	42–51	38 (†)	33–44	43.5	36.25–49.25

Data sharing the same symbols (*, †, ‡, §, ¶, **, ††, ‡‡, §§) differ significantly.
CODS, Clinical Oral Dryness Score; SPF, stimulated parotid flow; SWS, stimulated whole saliva flow; TA, triamcinolone acetonide; T0, 1 week before intervention; T1, 1 week after intervention; T8, 8 weeks after intervention; T16, 16 weeks after intervention; T24, 24 weeks after intervention; UWS, unstimulated whole saliva flow; XI, Xerostomia Inventory.

Between group analysis: saline group versus saline/TA group

No significant difference was found between these groups except for SPF at T24 (Mdn=0.34 mL/min, U=78, P=0.02, r=−0.40), which was significantly higher in the saline/TA group compared with SPF at T24 (Mdn=0.02 mL/min) in the saline group.

DISCUSSION

The results of our study indicate that sialendoscopy reduces oral dryness objectively and subjectively. Previous studies found that stricture formation is the major cause of obstruction of the salivary ducts and recurrent sialadenitis in patients with SS and other autoimmune diseases.[12 17] It has been suggested that removal of these strictures could improve salivary flow.[12 30] In this study, strictures were present and removed in all treated salivary glands.

We presume that improvement of salivary flow is only possible if saliva-producing acinar cells are present and functioning in the glandular tissue or when the parenchyma recovers. Therefore,

Table 3 Mean ESSPRI score (dryness domain) for all groups and time points

	Control group		95% CI		Saline group		95% CI		Saline/TA group		95% CI	
	Mean	SD	Lower limit	Upper limit	Mean	SD	Lower limit	Upper limit	Mean	SD	Lower limit	Upper limit
T0	8.00 (*)	1.13	7.37	8.63	6.88 (*)	1.66	5.99	7.76	7.92 (§§,¶¶,***)	1.52	7.16	8.67
T1	7.87 (†)	1.60	7.01	8.73	6.34 (†)	1.90	5.33	7.40	7.50 (†††,‡ ‡‡)	2.12	6.45	8.55
T8	8.20 (‡, §)	1.15	7.57	8.84	5.75 (‡)	1.98	4.69	6.81	6.22 (§§,†††,§)	1.92	5.26	7.18
T16	8.53 (¶,**)	1.19	7.88	9.19	6.22 (¶)	2.10	5.11	7.33	6.40 (¶¶,‡‡‡,**)	1.91	5.44	7.34
T24	8.01 (††,‡‡)	1.49	7.24	9.00	6.28 (††)	1.83	5.31	7.25	6.50 (***,‡‡)	2.26	5.28	7.72

Data sharing the same symbols (*, †, ‡, §, ¶, **, ††, ‡‡, §§, ¶¶, ***, †††, ‡‡‡) differ significantly (P<0.05).
ESSPRI, EULAR Sjögren's Syndrome Patient-Reported Index; TA, triamcinolone acetonide; T0, 1 week before intervention; T1, 1 week after intervention; T8, 8 weeks after intervention; T16, 16 weeks after intervention; T24, 24 weeks after intervention.

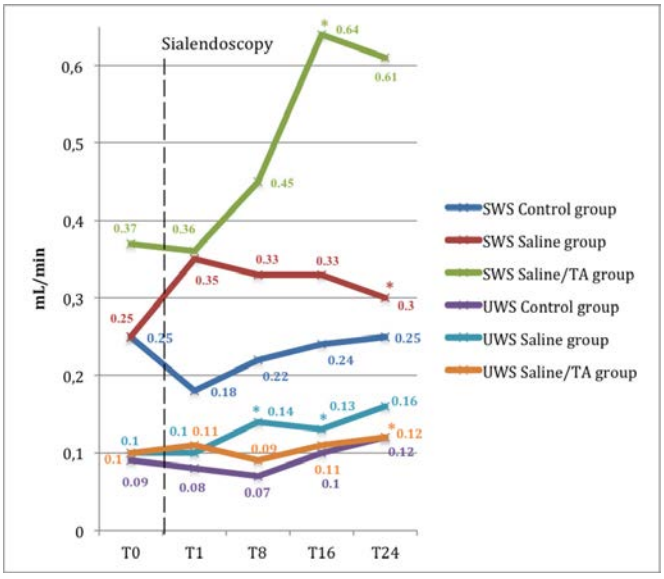

Figure 3 Change in median UWS and SWS before and after sialendoscopic rinsing. *P<0.05 compared with baseline (T0). SWS, stimulated whole saliva flow; TA, triamcinolone acetonide; T0, 1 week before intervention; T1, 1 week after intervention; T8, 8 weeks after intervention; T16, 16 weeks after intervention; T24, 24 weeks after intervention; UWS, unstimulated whole saliva flow.

the stage of the disease, the baseline level of stimulated salivary flow and the response of the glands to a stimulus are expected to have significant impact on the success of this treatment. It is possible that patients with recent onset of SS and more residual salivary gland capacity would benefit more from a sialendoscopic procedure than patients with long-standing disease. Ultrasound of the glands was not performed preoperatively, but might be useful to determine the stage of the disease in glands, thereby helping to identify which glands warrant sialendoscopy. In this study, the disease duration in the saline group was shorter than in the saline/TA group (8.1 and 11.1, respectively, table 1). As this difference was not significant, it could not fully explain differences between these groups in salivary flow after sialendoscopy.

Irrigation of the ductal system of the major salivary glands with saline/TA was not significantly more effective on SWS levels than irrigation with saline alone. It was expected that irrigation with saline/TA would have a larger effect than irrigating with only saline. Corticosteroids have anti-inflammatory effects, and inhibit T-cell activation. Salivary glands affected by SS are characterised by a focal periductal infiltrate consisting mainly of T lymphocytes and B lymphocytes.[31] Since the salivary gland duct directly connects to the gland, it could represent an effective route to deliver medications to the gland. However, it is questionable whether there is a large TA uptake by the tissues surrounding the duct during the relative short irrigating process. Another explanation for the larger effect of irrigating with saline/TA could be that in the saline/TA group the median baseline SWS level was higher compared with that in the saline group. Although this difference was not significant, it could be an explanation for the larger increase in SWS flow levels after sialendoscopy in the saline/TA group compared with the saline group suggesting a larger effect of sialendoscopy in patients with higher baseline salivary flow levels.

A complicating factor in this study was that it was not possible to identify or dilate the papilla to introduce the sialendoscope in all salivary glands. Along with the stage of the disease and baseline flow levels, this inconsistency could be a source of variation. It is possible that patients with more accessible glands benefited more from sialendoscopy than patients with blocked gland access. This study shows that mainly SPF improved after irrigation of the ductal system with saline/TA. This could also be explained by the accessibility of these glands. Sialendoscopy is more complicated to perform in submandibular glands affected by SS than in parotid glands. These anatomic conditions may impede the irrigating and the delivery of medication to the gland parenchyma.[18 32] Careful preoperative selection of patients and salivary glands could contribute to a higher percentage of successfully irrigated glands and more predictable results. Sialendoscopy of multiple salivary glands in the same session is safe and performing this procedure under local anaesthesia seems warranted.[33] In the saline/TA group, fewer submandibular glands (38.9%) could be irrigated compared with the saline group (56.3%). This could explain the smaller increase of UWS after sialendoscopy in the saline/TA group compared with the saline group. The follow-up period was too short to assess a long-term effect of sialendoscopy in patients with SS. Trials with a longer follow-up period are needed to prove the long-term sustainability of the observed effect of this treatment on salivary flow.

In our study, sialendoscopic intervention had a significant effect on the dryness domain of the ESSPRI and this result is partly supported by XI scores. This effect could be related to an increased flow, and to a change in salivary protein composition after sialendoscopy.[13] Improvement of the perceived oral dryness could also be related to a placebo effect, as it was not possible to perform the study as a double-blind randomised trial.

CONCLUSION

This randomised controlled trial assessed the effect of sialendoscopy of the major salivary glands on salivation and xerostomia in patients with SS. The results indicate that oral dryness improves up to 6 months after sialendoscopy, both subjectively and objectively, compared with baseline.

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Contributors KHK conceived the project, contributed to the study design, analysed and interpreted data, drafted the article and approved the final version for submission; AV interpreted the data, critically revised the article and approved the final version for submission; TF conceived the project, contributed to the study design, critically revised the article and approved the final version for submission; HSB contributed to the study design, critically revised the article and approved the final version for submission; FM collected data, critically revised the article and approved the final version for submission; DHJJ conceived and oversaw the project, contributed to the study design, analysed and interpreted data, drafted the article and approved the final version for submission.

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Competing interests None declared.

Patient consent Obtained.

Ethics approval The study was conducted in accordance with the ethical principles of the Declaration of Helsinki, International Council for Harmonisation on Good Clinical Practice and the applicable country-specific regulatory requirements. The VU University Medical Center Research Ethics Board (#NL44018.029.13) approved the study.

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Data sharing statement All data are deposited in the Dryad repository: https://doi.org/10.5061/dryad.r7m1n.

REFERENCES

1 Kroese FG, Abdulahad WH, Haacke E, *et al*. B-cell hyperactivity in primary Sjögren's syndrome. *Expert Rev Clin Immunol* 2014;10:483–99.
2 Thomas E, Hay EM, Hajeer A, *et al*. Sjögren's syndrome: a community-based study of prevalence and impact. *Br J Rheumatol* 1998;37:1069–76.
3 Fox RI. Sjögren's syndrome. *Lancet* 2005;366:321–31.
4 Baldini C, Talarico R, Tzioufas AG, *et al*. Classification criteria for Sjogren's syndrome: a critical review. *J Autoimmun* 2012;39:9–14.
5 Qin B, Wang J, Yang Z, *et al*. Epidemiology of primary Sjögren's syndrome: a systematic review and meta-analysis. *Ann Rheum Dis* 2015;74:1983–9.
6 Pijpe J, Kalk WW, Bootsma H, *et al*. Progression of salivary gland dysfunction in patients with Sjogren's syndrome. *Ann Rheum Dis* 2007;66:107–12.
7 Vissink A, Bootsma H, Kroese FG, *et al*. How to assess treatment efficacy in Sjögren's syndrome? *Curr Opin Rheumatol* 2012;24:281–9.
8 Ramos-Casals M, Brito-Zerón P, Font J. The overlap of Sjögren's syndrome with other systemic autoimmune diseases. *Semin Arthritis Rheum* 2007;36:246–55.
9 van Nimwegen JF, Moerman RV, Sillevis Smitt N, *et al*. Safety of treatments for primary Sjögren's syndrome. *Expert Opin Drug Saf* 2016;15:513–24.
10 Bootsma H, Kroese FGM, Vissink A. Editorial: rituximab in the treatment of Sjögren's Syndrome: is it the right or wrong drug? *Arthritis Rheumatol* 2017;69:1346–9.
11 Ramos-Casals M, Tzioufas AG, Stone JH, *et al*. Treatment of primary Sjögren syndrome: a systematic review. *JAMA* 2010;304:452–60.
12 Shacham R, Puterman MB, Ohana N, *et al*. Endoscopic treatment of salivary glands affected by autoimmune diseases. *J Oral Maxillofac Surg* 2011;69:476–81.
13 Jager DJ, Karagozoglu KH, Maarse F, *et al*. Sialendoscopy of salivary glands affected by Sjögren Syndrome: a randomized controlled pilot study. *J Oral Maxillofac Surg* 2016;74:1167–74.
14 Capaccio P, Canzi P, Torretta S, *et al*. Combined interventional sialendoscopy and intraductal steroid therapy for recurrent sialadenitis in Sjögren's syndrome: results of a pilot monocentric trial. *Clin Otolaryngol* 2018;43.
15 Atienza G, López-Cedrún JL. Management of obstructive salivary disorders by sialendoscopy: a systematic review. *Br J Oral Maxillofac Surg* 2015;53:507–19.
16 Maresh A, Kutler DI, Kacker A. Sialoendoscopy in the diagnosis and management of obstructive sialadenitis. *Laryngoscope* 2011;121:495–500.
17 Chuangqi Y, Chi Y, Lingyan Z. Sialendoscopic findings in patients with obstructive sialadenitis: long-term experience. *Br J Oral Maxillofac Surg* 2013;51:337–41.
18 Pace CG, Hwang KG, Papadaki M, *et al*. Interventional sialoendoscopy for treatment of obstructive sialadenitis. *J Oral Maxillofac Surg* 2014;72:2157–66.
19 Turner MD. Sialoendoscopy and salivary gland sparing surgery. *Oral Maxillofac Surg Clin North Am* 2009;21:323–9.
20 McGurk M. Commentary on: management of obstructive salivary disorders by sialendoscopy: a systematic review. *Br J Oral Maxillofac Surg* 2015;53:520–1.
21 Vitali C, Bombardieri S, Jonsson R, *et al*. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis* 2002;61:554–8.
22 Osailan SM, Pramanik R, Shirlaw P, *et al*. Clinical assessment of oral dryness: development of a scoring system related to salivary flow and mucosal wetness. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2012;114:597–603.
23 Thomson WM, Chalmers JM, Spencer AJ, *et al*. The Xerostomia Inventory: a multi-item approach to measuring dry mouth. *Community Dent Health* 1999;16:12–17.
24 Seror R, Bootsma H, Saraux A, *et al*. Defining disease activity states and clinically meaningful improvement in primary Sjögren's syndrome with EULAR primary Sjögren's syndrome disease activity (ESSDAI) and patient-reported indexes (ESSPRI). *Ann Rheum Dis* 2016;75:382–9.
25 Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: updated guidelines for reporting parallel group randomized trials. *Ann Intern Med* 2010;152:726–32.
26 Navazesh M. Methods for collecting saliva. *Ann N Y Acad Sci* 1993;694:72–7.
27 Kalk WW, Vissink A, Spijkervet FK, *et al*. Sialometry and sialochemistry: diagnostic tools for Sjögren's syndrome. *Ann Rheum Dis* 2001;60:1110–6.
28 Jager DHJ, Bots CP, Forouzanfar T, *et al*. Clinical oral dryness score: evaluation of a new screening method for oral dryness. *Odontology* 2018.
29 Dupont WD, Plummer WD. Power and sample size calculations. A review and computer program. *Control Clin Trials* 1990;11:116–28.
30 De Luca R, Trodella M, Vicidomini A, *et al*. Endoscopic management of salivary gland obstructive diseases in patients with Sjögren's syndrome. *J Craniomaxillofac Surg* 2015;43:1643–9.
31 Izumi M, Eguchi K, Nakamura H, *et al*. Corticosteroid irrigation of parotid gland for treatment of xerostomia in patients with Sjögren's syndrome. *Ann Rheum Dis* 1998;57:464–9.
32 Marchal F, Dulguerov P, Becker M, *et al*. Submandibular diagnostic and interventional sialendoscopy: new procedure for ductal disorders. *Ann Otol Rhinol Laryngol* 2002;111:27–35.
33 Karagozoglu KH, De Visscher JG, Forouzanfar T, *et al*. Complications of sialendoscopy in patients with Sjögren Syndrome. *J Oral Maxillofac Surg* 2017;75:978–83.

EXTENDED REPORT

Common measure of quality of life for people with systemic sclerosis across seven European countries: a cross-sectional study

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ABSTRACT

Objectives The aim of this study was to adapt the Systemic Sclerosis Quality of Life Questionnaire (SScQoL) into six European cultures and validate it as a common measure of quality of life in systemic sclerosis (SSc).

Methods This was a seven-country (Germany, France, Italy, Poland, Spain, Sweden and UK) cross-sectional study. A forward–backward translation process was used to adapt the English SScQoL into target languages. SScQoL was completed by patients with SSc, then data were validated against the Rasch model. To correct local response dependency, items were grouped into the following subscales: function, emotion, sleep, social and pain and reanalysed for fit to the model, unidimensionality and cross-cultural equivalence.

Results The adaptation of the SScQoL was seamless in all countries except Germany. Cross-cultural validation included 1080 patients with a mean age 58.0 years (SD 13.9) and 87% were women. Local dependency was evident in individual country data. Grouping items into testlets corrected the local dependency in most country specific data. Fit to the model, reliability and unidimensionality was achieved in six-country data after cross-cultural adjustment for Italy in the social subscale. The SScQoL was then calibrated into an interval level scale.

Conclusion The individual SScQoL items have translated well into five languages and overall, the scale maintained its construct validity, working well as a five-subscale questionnaire. Measures of quality of life in SSc can be directly compared across five countries (France, Poland Spain, Sweden and UK). Data from Italy are also comparable with the other five countries although require an adjustment.

INTRODUCTION

Systemic sclerosis (SSc) is a heterogeneous connective tissue disease characterised by vasculopathy, immune activation and fibrosis.[1–3] The multisystem involvement in the disease has severe physical and psychosocial impact affecting the patients' quality of life (QoL). QoL is a complex interaction between the ways in which people perceive their health and how it relates to other aspects of their lives that are less directly health-specific.

Several tools have been used in different studies to capture QoL in people with SSc, such as the SF-36 and the EuroQol 5-Domain health questionnaire,[4–6] however, these tools are not disease-specific and can be less sensitive to the more directly disease-related factors. To capture the true psychosocial impact of the disease, a needs-based disease-specific QoL is the gold standard. The Systemic Sclerosis Quality of Life Questionnaire (SScQoL), developed by Reay[7] and translated into six languages in this paper, was developed according to this principle. The SScQoL tool measures the disease impact on health and well-being, and has been developed using a needs-based quality of life model, which is based on the understanding that individuals are driven or motivated by their needs and that life gains its quality from the ability and capacity of individuals to satisfy their needs.[8 9]

During its development, the original SScQoL[7] was subject to strict principles of item response theory to ensure the highest quality measure of needs-based patient-reported QoL reporting in people with SSc. The SScQoL joins a stable of measures including the Rheumatoid Arthritis Quality of Life,[10] Osteoarthritis Quality of Life[11] and Ankylosing Spondylitis Quality of Life[12] developed at the University of Leeds and forming the cornerstone of patient-reported outcome measurement (PROM) in many rheumatological conditions.

The SScQoL is a self-completed questionnaire comprising 29 questions exploring the impact of SSc on health and well-being, covering four themes identified by patients with SSc: emotion, physical adaptation, impact on/with others and impact on self. It takes the patient approximately 5 mins to complete and provides quantitative data that enables the health professional involved to accurately evaluate the impact of SSc on an individual patient or groups of people with the disease. Due to its robust validation, the SScQoL can also be used with confidence as a research tool to evaluate pharmacological and non-pharmacological interventions.

Initial development and testing demonstrated the reliability, validity and the patient acceptance of the instrument and the original English language version of the tool has been subjected to Rasch

Analysis to ensure its construct validity, unidimensionality and absence of differential item functioning.[7]

The relevance of a common measure

SSc is considered a rare disease due to its prevalence (82 per 1 000 000 adjusted for the UK population).[13] The small numbers of people affected by SSc causes methodological problems, particularly when developing research studies requiring large sample sizes. To overcome these problems, there is a need for multicentre and international studies, using common outcome measures, which have demonstrable cross-cultural relevance and measurement equivalence, which in turn allow researchers to obtain reliable results that are comparable across countries. In addition, the existing European collaborations and networks such as EUSTAR and EUSHNet can employ a common measure prospectively in a systematic way, such that the networks and patients in the countries involved can benefit from the consistency provided by a cross-culturally valid measure.

The objectives of this study therefore were to: (i) translate and adapt the SScQoL for use in Germany, France, Italy, Poland, Spain and Sweden; (ii) undertake a cross-cultural validation of the SScQoL for use in these countries; (iii) calibrate a common scale that is comparable across countries and (iv) ultimately incorporate the translated and validated version of the SScQoL into the EUSTAR MEDS database to create a common minimum dataset for PROMS in SSc research in Europe. This paper reports the results of the objectives (i) through (iii).

METHODS

Study design

This was a multicentre cross-sectional analytic study involving seven European countries; Germany, France, Italy, Poland, Spain, Sweden and the UK. The study involved two phases (i) cross-cultural adaptation and (ii) cross-cultural validation.

Cross-cultural adaptation phase

The English SScQoL was adapted into six languages using the well-established process of cross-cultural adaptation of self-report measures.[14] The aim of cross-cultural adaptation is to ensure conceptual equivalence between original and target versions of a questionnaire. This process involved the following stages for each of the collaborating centres: (i) the original (English) version was translated into the target language by two translators working independently; (ii) the translations were compared and any inconsistencies resolved; (iii) the translated tools were then translated back into English by a translator not involved in stage one; (iv) once satisfactory translations had evolved, all four versions were reviewed by an expert committee and any outstanding inconsistencies were resolved by discussion and (v) the adapted questionnaires were completed by 30 patients with SSc in each of the collaborating centres.

Cross-cultural validation phase

The aim of this stage was to ensure measurement equivalence of the SScQoL, to enable common measurement across the seven countries. During this phase, the translated questionnaires were completed by 100–270 patients (in each country) by either postal or site survey. Participants were native speakers of the target languages except in Sweden where seven participants were non-native but all had lived in Sweden for several years and had a good ability to speak and read Swedish. The data from the new SScQoL were then subjected to Rasch analysis, which involved testing the construct validity of each translated tool, internal consistency and the cross-cultural invariance of the tool across all the seven countries. Finally, the common measure was calibrated, which takes account of cultural differences and, if successful, provides for pooling and comparison of measurements across the various culturally adapted versions.

Patients

Each centre recruited a convenience sample of patients from rheumatology outpatient clinics and/or patient databases. The inclusion criteria were: (i) consultant diagnosis of SSc according to ARA/ACR 1980 criteria,[15] (ii) aged ≥18 years and (iii) willingness and ability to complete and return a questionnaire. The only exclusion criterion was an inability to understand or complete the written questionnaire. Participation in the study was voluntary and each of the collaborating centres followed ethical procedures applicable to their respective countries before recruiting patients. Local investigators in each collaborating country handled all patients' (interview) data collected during the cross-cultural adaptation phase. The data collected during the validation phase in each country was then sent to the University of Leeds for psychometric testing using Rasch models. Data transferred were limited to anonymised SScQoL data containing patient's age and gender information.

Data analysis

The validation data were analysed using RUMM2030 software (RUMM Laboratory, Perth, Western Australia). First, each country-specific dataset was tested for fit to the Rasch model. Fit to the Rasch model implies construct validity, reliability, unidimensionality and statistical sufficiency of the total score from the scale.[16–19] Model fit was determined by item–person interaction statistics which compare the difference between observed responses and values expected by the model (standardised residuals). The following statistics suggest fit to the model: (i) item–person interaction statistics, distributed as a Z statistic with a mean of 0 and SD of 1; (ii) item χ^2 statistic (comparing the difference between observed and expected values) with a non-significant probability—several χ^2 are computed for each item across groups, therefore Bonferroni adjustment is required to avoid type I errors due to multiple testing;[20] (iii) item–trait interaction statistic reported as a non-significant χ^2 probability, reflecting the invariance of the SScQoL to different levels of quality of life.

An estimate of internal consistency (reliability) was determined by person separation index (PSI), which represents the ability of the SScQoL to distinguish between people with different levels of reported quality of life. A value of 0.7 is required for group use.[19]

Although fit to Rasch model implies unidimensionality of the scale, further tests were carried out to confirm the assumption of local independence of items,[21] unidimensionality and differential item functioning. The Rasch model assumes that each item independently contributes to the underlying construct, no significant item–item residual correlations are expected therefore, after contribution to the construct is removed. Where significant item–item residual correlations were identified (through residual correlation matrices), these locally dependent items were grouped and treated as a unit, referred to as a 'testlet', which represent a subscale. Two investigators (MN and ACR) grouped the items by consensus into the following testlets: function, emotion, sleep, social and pain, which in turn map onto the International Classification

of Functioning, Disability and Health model. The testlets were treated as 'superitems' in the subsequent analyses.

Unidimensionality was confirmed using the principal component analysis and t-test-based method proposed by Smith.[22] Two sets of items hypothesised to represent low levels and high levels of quality of life were defined, based on the correlation between items and the first residual factor. An independent t-test was then used to compare the difference in these estimates for each person. Unidimensionality was confirmed if ≤5% of the t-tests were significant or if lower bound of a binomial 95% CI of the observed proportion overlapped 5%.[19 22]

Cross-cultural (measurement) equivalence was tested using the differential item functioning (DIF) analysis feature in-built into RUMM2030. This is based on a two-way analysis of variance (ANOVA) of residuals across each level of person factor (in this case, culture) and across different levels of trait (in this case, quality of life). Presence of uniform DIF was suggested if the P value of the main effects (culture) was significant. This test flags the presence of significant DIF in the pooled datasets (significant difference between two or more group means) but does not specify where the difference lies. The post hoc Tukey test which performs a pairwise comparison of means was used to explore DIF patterns and identify which country-specific dataset(s) exhibited the DIF. Once identified, the testlet affected by cross-cultural DIF was 'split' into two, to provide a culturally specific (emic) testlet for the country exhibiting the DIF and a culturally general (etic) testlet for the rest of the countries. Once the DIF-affected testlet was split, the pooled data were reanalysed to assess fit to the model. This method of post hoc DIF analysis is detailed elsewhere.[23–26]

When fit to the model was established, the raw SScQoL scores were mapped against the corresponding Rasch-transformed (logit-based) scores and were linearly transformed to calibrate an interval scale of the same range. This allows for transformation of raw scores to interval scaling.[27] The raw-to-linear score conversion table provided the adjustment for the cross-cultural difference via the split testlet.[23–25 27]

RESULTS

Cross-cultural adaptation

The adaptation of the SScQoL into European languages was largely seamless except for the German dataset in which patients had reported problems in providing strictly dichotomous 'yes/no' responses on the following 10 items: (Q4) my condition makes me angry; (Q9) my condition means I have disturbed sleep; (Q11) it has affected the health of people around me; (Q12) my hands do not work as well as they did; (Q13) it puts a strain on my personal relationships; (Q15) any sort of activity is difficult; (Q19) I cannot cope at all; (Q20) sleeping badly has affected me a lot; (Q25) I struggle to wash myself as I would like; (Q27) I feel helpless and (Q29) I miss being able to sort things out. In Sweden, patients reported problems with two items: with regard to (Q5) 'I get upset when I cannot do things' they preferred using 'disappointed' or 'sad' instead of 'upset' and for (Q10) 'it has affected me a lot socially', participants suggested to remove 'a lot'.

For the Spanish translation, in item Q27 (I feel helpless), the translators had difficulties in finding a word that captured the English meaning of 'helpless' ('impotencia' in Spanish). A consensus was reached among the translators that the Spanish word 'impotencia' which means 'impotence' in English had the closest meaning to 'helpless/powerlessness'. Since 'impotencia' also means sexual dysfunction, translators recommended that clarification should be provided to the patients when the questionnaire is issued to avoid confusion.

Table 1 Sample characteristics by country

	Sample	Gender		Age	
Country	N	M (%)	F (%)	Mean	SD
UK	121	15 (12.40)	106 (87.60)	57.09	12.073
France	115	18 (15.65)	97 (84.35)	59.05	13.226
Italy	131	16 (12.31)	114 (87.69)	57.96	15.031
Sweden	102	9 (8.74)	94 (91.26)	60.01	12.332
Germany	274	27 (9.90)	239 (87.20)	60.84	10.569
Poland	231	33 (14.29)	198 (85.71)	55.85	12.552
Spain	106	19 (17.92)	87 (82.08)	54.84	13.971
Pooled	1080	137 (12.69)	943 (87.31)	57.95	13.894

Validation

Patient characteristics

In total, 1080 patients were recruited and their age and gender distribution parameters are summarised in table 1.

Fit to the Rasch model

Table 2 presents item–person fit statistics reliability and unidimensionality of the SScQoL for individual countries' datasets. The initial analyses of the 29-item scales for each country (based on individual items, table 2A) suggest an initial lack of fit for the German, Italian, Polish and Swedish data (values representing a perfect fit to the model are given in the lowest row of table 2). Individual item fit statistics for each country are provided in the online supplementary table S1 . Assessment of the residual correlation matrix revealed significant local dependence (item–item residual correlation >0.3), which was largely responsible for the lack of fit.

The 29 items of the full scale were the mapped by consensus by the project leaders (MN and ACR) onto five domains corresponding to the components of the International Classification of Functioning, Disability and Health model: function (activity limitation), emotional (personal factors), sleep (personal factors), social (participation restrictions) and pain (impairment) (see table 3).

Using the 5-testlet model, the responses for six countries (France, Italy, Poland, Spain, Sweden and the UK) showed a good fit to the Rasch model confirming construct validity, reliability and unidimensionality in the country-specific data (see table 2B). The German data continued to exhibit significant deviations from the Rasch model (item residual mean −0.698, SD 2.795, item–trait interaction χ^2 P<0.001). The datasets for the six countries that had evidence of fit to the Rasch model (France, Italy, Poland, Spain, Sweden and the UK) were combined in a pooled analysis and the results suggested that each testlet had an acceptable fit to the Rasch model (see table 2B). However, the item–trait (testlet–trait) interaction χ^2 statistic for the pooled dataset continued to display significant deviation from the model expectations (χ^2=63.909, df=45, P=0.034) suggesting lack of invariance (presence of DIF) across different levels of quality of life.

Cross-cultural invariance

DIF analysis highlighted a significant cross-cultural bias in the social subscale (table 4). Post hoc Tukey analysis revealed that the DIF was displayed by the Italian dataset. The social subscale

Table 2 Item–person fit statistics, reliability and unidimensionality of the SScQoL (country-specific data)

	Item fit residual		Person fit residual		Item–trait χ^2 interaction		Person separation reliability		Unidimensionality test*
Country	Mean	SD	Mean	SD	Value (DF)	P value	PSI	N	Proportion of independent t-tests (binomial 95% CI)
A: Analysis of the SScQoL with individual 29 items									
UK	−0.232	1.043	−0.233	0.680	48.932 (29)	0.012	0.919	112	0.099 (0.060 to 0.138)
France	−0.291	1.048	−0.271	0.855	34.868 (29)	0.209	0.893	111	0.087 (0.047 to 0.127)
Germany	−0.704	2.034	−0.484	1.456	398 (116)	**<0.001**	0.881	263	0.063 (0.028 to 0.099)
Italy	−0.205	1.051	−0.285	0.756	88.662 (58)	**0.006**	0.890	125	0.053 (0.016 to 0.091)
Poland	−0.520	1.380	−0.342	0.950	187.494 (116)	**<0.001**	0.902	221	0.099 (0.060 to 0.138)
Spain	−0.166	0.751	−0.220	0.654	42.439 (29)	0.051	0.906	95	0.075 (0.033 to 0.116)
Sweden	−0.264	0.910	−0.273	0.728	60.886 (29)	**<0.001**	0.892	101	0.068 (0.026 to 0.110)
B: Analysis of the SScQoL as a 5-testlet scale									
UK	0.016	1.535	−0.288	0.848	4.992 (5)	0.417	0.896	109	0.050 (0.011 to 0.088)
France	0.020	1.380	−0.280	0.895	2.368 (5)	0.796	0.826	106	0.028 (-0.013 to 0.068)
Germany	−0.698	2.795	−0.285	0.873	61.952 (20)	**<0.001**	0.852	263	0.030 (-0.013 to 0.073)
Italy	−0.137	1.888	−0.315	0.831	4.281 (5)	0.510	0.818	125	0.038 (0.001 to 0.075)
Poland	−0.348	2.139	−0.308	0.966	22.450 (15)	0.096	0.853	221	0.043 (0.015 to 0.071)
Spain	−0.265	1.346	−0.221	0.711	11.430 (5)	0.043	0.846	92	0.018 (-0.023 to 0.060)
Sweden	−0.136	0.793	−0.253	0.819	25.665 (44)	0.988	0.813	95	0.030 (-0.013 to 0.073)
Perfect fit	0	1	0	1		>0.05	>0.70		≤0.05 or lower-bound 95%CI≤0.05

P value, χ^2 probability, where >0.05 (>0.01 for Bonferroni correction) suggest adequate fit to the model.
*Unidimensionality was deemed supported if ≤5% (0.05) of independent t-tests were significant or if lower bound of a binomial 95% CI of the observed proportion overlapped 5% (0.05).
DF, degree of freedom; PSI, Person Separation Index (internal consistency) reliability; SScQoL, Systemic Sclerosis Quality of Life Questionnaire.

was therefore 'split' such that there was a social-etic subscale which is culturally general (for five countries—France, Poland, Spain, Sweden and the UK) and a social-emic subscale which was culturally specific to Italy. This split improved the overall fit statistics of the pooled data (see the online supplementary table S2). The subsequent item–trait χ^2 statistic suggested adequate fit to the model (χ^2=65.580, df=54, P=0.140) and the reliability remained good (PSI=0.841).

Calibrating an interval scale

Following DIF analysis and the adjustment for cross-cultural DIF, the raw scale scores were transformed into logit-based (interval level) scores for the five testlets, with an adjusted social subscale for Italy (see table 5).

DISCUSSION

The original SScQoL was developed with patients to ensure it captures HRQoL aspects that are of interest to patients. Having satisfied the requirements of the Rasch model expectations, the tool has demonstrated validity, reliability and statistical sufficiency.[7] In this study, a new UK dataset was collected and the conclusions were consistent with those of the original development study. Additionally, we have employed a standardised method of questionnaire adaptation into the European languages (and associated cultures), ensuring that the tool maintains a conceptual equivalence between the original and the adapted (translated) versions. Furthermore, in this analysis, we have transposed the concept of SScQoL into five subscale measures, where each item contributes to the subscale and the overall dimension. Therefore, the subscale score provides the estimate of quality of life specific for that domain (function, emotional, sleep and so on) and the total score provides a sufficient statistic for overall health-related quality of life in SSc.

Table 3 Testlets formed by grouping items

Testlet	Number of items	Items
Function	6	1, 12, 14, 15, 22 and 25
Emotional	13	2, 3, 4, 5, 6, 7, 8, 17, 18, 19, 24, 27 and 29
Sleep	2	9 and 20
Social	6	10, 11, 13, 16, 21 and 23
Pain	2	26 and 28

Rasch analyses confirmed measurement equivalence between the English and all adapted versions except the German, where patients had found it difficult to complete some dichotomous items, indicating a preference for rating scales or having more options. This implies that for those patients, the dichotomous items, as presented, failed to capture the full range of their responses. As the SScQoL is a needs-based measure, failure of the tool to measure the full range of patient responses appears to have had an impact on the validity of the German version of the scale as reflected in the lack of fit to the model. Future work on this specific version will explore the wording and presentation of the root questions, as well as the potential for, and impact of making available trichotomous or higher level response options.

All translations, except the German version, demonstrated sufficient validity and fit to the Rasch model to support their use for single-country studies and within-country comparisons. The current analysis confirmed the unidimensionality and measurement equivalence in five of the six countries when evaluated across five domains. Good internal validity and reliability ensures that clinicians (and patients) can use the tool with confidence when evaluating HRQoL at person and at population levels. Using the SScQoL alongside other outcome measures ensures that quality of life is being taken into account when providing care.

Since comparable scales are now available for six of the seven languages/cultures, employing the logit-based transformed scores as summarised in table 4 enables accurate estimation of SScQoL as an interval level measure, as well as comparability across cultures.

Table 4 Results of DIF analysis in pooled data (France, Italy, Poland, Spain, Sweden and UK)

	Main effects: country (uniform DIF)				Interaction effects: class interval by country (non-uniform DIF)			
Testlet	Mean square	F-statistic	Degrees of freedom	P value*	Mean square	F-statistic	Degrees of freedom	P value*
Function	1.736	2.711	5	0.030	1.033	1.613	36	0.015
Emotional	1.777	3.297	5	0.011	0.574	1.065	36	0.371
Sleep	3.832	3.264	5	0.012	0.961	0.818	36	0.766
Social	9.603	15.839	5	**<0.001**	0.706	1.165	36	0.240
Pain	1.627	1.680	5	0.153	0.978	1.010	36	0.455

*Significant Bonferroni adjusted P value ≤0.003 suggest presence of uniform DIF.
DIF, differential item functioning.

This means that it is now possible to pool large datasets across countries and/or to develop collaborative projects using a common measure of SScQoL. It is recommended therefore that future studies report subscale scores routinely, as well as reporting single-scale scores, to facilitate comparison and data pooling across countries. As further work will be required to explore how the German SScQoL works in other samples, caution will be required until this work is complete when comparing between German scores and scores from other countries.

This study sets out to establish a common measure of QoL in SSc across seven countries. The study has two main limitations. First, the cross-cultural validation of the SScQoL in Germany did not work as expected. Two subsets of data were collected for this analysis and these datasets, both individually and in pooled

Table 5 Conversion table for raw-to-linear (Rasch transformed) scores with cross-cultural adjustment

Raw scores (yes=1, no=0)	Function (all)	Emotional (all)	Sleep (all)	Social (Italy)	Social (others)	Pain (all)	Total (Italy)	Total (others)
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1.0	1.3	1.6	1.0	0.8	1.6	1.0	2.9	3.6
2.0	2.4	2.8	2.0	1.4	2.6	2.0	4.8	6.0
3.0	3.1	3.7		1.8	3.3		6.0	7.5
4.0	3.8	4.5		2.4	3.9		6.9	8.6
5.0	4.8	5.2		3.7	4.7		7.7	9.6
6.0	6.0	5.9		6.0	6.0		8.3	10.3
7.0		6.6					8.9	11.0
8.0		7.3					9.4	11.6
9.0		8.0					9.9	12.2
10.0		8.9					10.3	12.8
11.0		9.9					10.8	13.3
12.0		11.2					11.2	13.8
13.0		13.0					11.6	14.3
14.0							12.1	14.8
15.0							12.5	15.3
16.0							12.9	15.8
17.0							13.4	16.3
18.0							13.9	16.8
19.0							14.3	17.3
20.0							14.8	17.8
21.0							15.3	18.4
22.0							15.9	19.0
23.0							16.5	19.7
24.0							17.1	20.4
25.0							18.0	21.3
26.0							19.0	22.4
27.0							20.6	23.8
28.0							23.6	25.9
29.0							29.0	29.0

The SScQoL has dichotomous yes/no responses, coded as 1 (yes) and 0 (no), yielding a scoring range 0–6 for the function subscale, 0–13 for the emotional subscale, and so on. The scores obtained from the patient are the raw scores and these must be converted to linear scores using the conversion chart. For example, if a patient has a raw score of 2 on the functional subscale, this will be transformed to 2.4, if the patient has a raw scores of 3 on the emotional subscale this will transformed to 3.7, and so on in the other subscales. The social subscale is split, with transformed scores for Italy and the rest of the countries. If a patient from Italy has a raw score of 4 on the social subscale, this will be transformed to 2.4, but a raw score of 4 from patients in other countries will be transformed to 3.9. Adding up all the transformed subscale scores gives the total SScQoL score which is a comparable estimate of the patient's quality of life (range 0–29), higher scores indicating a worse quality of life.
Others=France, Poland, Spain, Sweden and the UK.
SScQoL, Systemic Sclerosis Quality of Life Questionnaire.

form, showed lack of fit the model. Further work is required to explore different ways of formatting the items in such a way that a full range of patient responses will be better captured. Second, ethics committees in some countries permitted collecting only basic demographic details (age and gender) in addition to the SScQoL items, and this may have limited the factors or subgroups being tested for invariance. Third, being a cross-sectional study, this study did not assess the sensitivity to change of the adapted versions. Sensitivity to change was established for the original (English) version, and it is expected that the adapted versions will also demonstrate this. Further research will be required to determine the minimal clinically important difference to support measurement of the impact or of treatments on the quality of life in people with SSc. As result of the successful cross-cultural validation of the SScQoL into six different European countries, we recommend for this tool to be translated into more European languages and to be adopted as part of a core set of tools used in SSc observational and clinical trials studies. An implementation phase, working in combination with colleagues within the EUSTAR network and beyond, is required to move towards a more systematic approach to clinical data capture in SSc research.

CONCLUSION

The individual SScQoL items have translated well into five European languages and overall, the scale maintained its construct validity, working well as a five-subscale questionnaire. Using the logit-based transformed scores, measures of quality of life in SSc can be directly compared across five countries (France, Poland, Spain, Sweden and UK). Data from Italy are also comparable with the other five countries using a separate adjusted scale, which sufficiently recalibrates the scores in the social subscale, so as to allow a valid comparison across countries. While comparison between German scores and the other countries will need further testing, it is likely that this can be accomplished with some extra work and in the interim, this study has provided a common measure of quality of life in people with SSc across six European countries. Future work will be required to define the thresholds of health-related quality of life and clinically meaningful change in SSc and to further adapt the SScQoL into a wider range of languages and cultural settings. Different versions of the SScQoL can be obtained at: https://doi.org/10.5518/325.[28]

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Contributors AR (professor of clinical biomechanics) is the principal investigator, designed the study, led the grant application, oversaw the project and undertook the statistical analyses, interpretation of the results and revised the study report for intellectual content. MN (senior lecturer in rheumatology nursing) codesigned the study with AR and BA-P, drafted the statistical analysis plan, contributed to the grant application, coordinated the project, undertook the statistical analyses, interpretation of the results and drafted the study report and revised it for intellectual content. BA-P (clinical post doctoral research fellow) was a member of the study team, a co-applicant on the study grant, contributed to the drafting of the manuscripts and revised it for intellectual content. YA (professor of rheumatology, rheumatologist) led the study team in France, contributed to the drafting of the study report and revised it for intellectual content. FDG (associate professor, head of scleroderma programme, rheumatologist) was a member of the study team, a co-applicant on the study grant, contributed to the drafting of the manuscripts and revised it for intellectual content. MF (resident physician and biostatistician) was a member of the study team in Germany, contributed to the translation of the SScQoL, data collection and drafting and revising the manuscripts for intellectual content. SG-D (rheumatology nurse specialist) led the study team in Spain, contributed to the drafting of the manuscripts and revised it for intellectual content. RH (associate professor of rheumatologist) was a member of the study team in Sweden, contributed to the drafting of the manuscripts and revised it for intellectual content. CK (physician assistant) was a member of the study team in Germany, contributed to the drafting of the manuscripts and revised it for intellectual content. MM-C (professor of rheumatology and medicine) led the study team in Italy, contributed to the drafting of the manuscripts and revised it for intellectual content. UM-L (professor of rheumatology) led the study team in Germany, contributed to the drafting of the study report and revised it for intellectual content. GS (associate professor, rheumatology occupational therapist) led the study team in Sweden, contributed to the drafting of the manuscripts and revised it for intellectual content. VT-S (consultant rheumatologist) was a member of the study team in Spain, contributed to the drafting of the manuscripts and revised it for intellectual content. TS (consultant rheumatologist) was a member of the study team in Germany, responsible for data collection in Germany. He contributed to the drafting of the manuscripts and revised it for intellectual content. MS (senior lecturer in nursing) led the study team, adaptation of the SScQoL and data collection in Poland and contributed to the drafting of the study report and revised it for intellectual content. JS (english instructor) was a member of the study team in Poland, responsible for the translation process of the SScQoL, contributed to the drafting of the study report and revised it for intellectual content. SS (professor of rheumatology) was a member of the study team in Poland, responsible for data collection for the validation of the SScQoL, contributed to the drafting of the study report and revised it for intellectual content. All authors read and approved the final version.

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Patient consent Detail has been removed from this case description/these case descriptions to ensure anonymity. The editors and reviewers have seen the detailed information available and are satisfied that the information backs up the case the authors are making.

Ethics approval This was a multicentre study conducted in seven countries (Germany, France, Italy, Poland, Spain, Sweden and UK) and was approved by local ethical committees or institutional review boards in each participating country.

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REFERENCES

1 Johnson SR, Fransen J, Khanna D, *et al*. Validation of potential classification criteria for systemic sclerosis. *Arthritis Care Res* 2012;64:358–67.
2 Abraham DJ, Varga J. Scleroderma: from cell and molecular mechanisms to disease models. *Trends Immunol* 2005;26:587–95.
3 Geyer M, Müller-Ladner U. The pathogenesis of systemic sclerosis revisited. *Clin Rev Allergy Immunol* 2011;40:92–103.
4 Strickland G, Pauling J, Cavill C, *et al*. Predictors of health-related quality of life and fatigue in systemic sclerosis: evaluation of the EuroQol-5D and FACIT-F assessment tools. *Clin Rheumatol* 2012;31:1215–22.
5 Gualtierotti R, Scalone L, Ingegnoli F, *et al*. Health related quality of life assessment in patients with systemic sclerosis. *Reumatismo* 2010;62:210–4.
6 Hudson M, Thombs BD, Steele R, *et al*. Health-related quality of life in systemic sclerosis: a systematic review. *Arthritis Rheum* 2009;61:1112–20.
7 Reay N. *The quality of life in patients with diffuse and limited systemic sclerosis [Monograph*: University of Leeds, 2008.
8 McKenna SP, Doward LC. The needannrheumdis-2017-212412s-based approach to quality of life assessment. *Value Health* 2004;7(s1):S1–3.
9 Maslow AH, Frager R, Fadiman J, *et al*; *Motivation and personality*. Harper & Row New York, 1970.
10 Tijhuis GJ, de Jong Z, Zwinderman AH, *et al*. The validity of the Rheumatoid Arthritis Quality of Life (RAQoL) questionnaire. *Rheumatology* 2001;40:1112–9.
11 Keenan AM, McKenna SP, Doward LC, *et al*. Development and validation of a needs-based quality of life instrument for osteoarthritis. *Arthritis Rheum* 2008;59:841–8.
12 Doward LC, Spoorenberg A, Cook SA, *et al*. Development of the ASQoL: a quality of life instrument specific to ankylosing spondylitis. *Ann Rheum Dis* 2003;62:20–6.
13 Allcock RJ, Forrest I, Corris PA, *et al*. A study of the prevalence of systemic sclerosis in northeast England. *Rheumatology* 2004;43:596–602.
14 Beaton DE, Bombardier C, Guillemin F, *et al*. Guidelines for the process of cross-cultural adaptation of self-report measures. *Spine* 2000;25:3186–91.
15 Masi AT. Subcommittee For Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Preliminary criteria for the classification of systemic sclerosis (scleroderma). *Arthritis & Rheumatism* 1980;23:581–90.
16 Bond TG, Fox CM. *Applying the Rasch model: Fundamental measurement in the human sciences*. London: Lawrence Arlbaum Associates, 2001.
17 Rosenbaum PR. Criterion-related construct validity. *Psychometrika* 1989;54–625–33.
18 Andersen EB. Sufficient statistics and latent trait models. *Psychometrika* 1977;42:69–81.
19 Tennant A, Conaghan PG. The Rasch measurement model in rheumatology: what is it and why use it? When should it be applied, and what should one look for in a Rasch paper? *Arthritis Rheum* 2007;57:1358–62.
20 Bland JM, Altman DG. Multiple significance tests: the Bonferroni method. *BMJ* 1995;310:170.
21 Lord FM, Novick MR, Birnbaum A. *Statistical theories of mental test scores*, 1968.
22 Smith EV. Detecting and evaluating the impact of multidimensionality using item fit statistics and principal component analysis of residuals. *J Appl Meas* 2002;3:205–31.
23 Tennant A, Penta M, Tesio L, *et al*. Assessing and adjusting for cross-cultural validity of impairment and activity limitation scales through differential item functioning within the framework of the Rasch model: the PRO-ESOR project. *Med Care* 2004;42:I–37.
24 Lange R, Thalbourne MA, Houran J, *et al*. Depressive Response Sets due to gender and culture-based Differential Item Functioning. *Pers Individ Dif* 2002;33:937–54.
25 Hagquist C, Andrich D. Recent advances in analysis of differential item functioning in health research using the Rasch model. *Health Qual Life Outcomes* 2017;15:181.
26 Ndosi M, Bremander A, Hamnes B, *et al*. Validation of the educational needs assessment tool as a generic instrument for rheumatic diseases in seven European countries. *Ann Rheum Dis* 2014;73:2122–9.
27 Wright BD, Linacre JM. Observations are always ordinal; measurements, however, must be interval. *Arch Phys Med Rehabil* 1989;70:857–60.
28 Redmond A, Tennant A, Horton M, *et al*. *Systemic Sclerosis Quality of Life (SScQoL) instrument: translation and cross cultural validation across seven European countries (UK, France, Italy, Spain, Sweden, Germany and Poland)*. University of Leeds, 2018. [Dataset].

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EXTENDED REPORT

MR-PheWAS: exploring the causal effect of SUA level on multiple disease outcomes by using genetic instruments in UK Biobank

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ABSTRACT

Objectives We aimed to investigate the role of serum uric acid (SUA) level in a broad spectrum of disease outcomes using data for 120 091 individuals from UK Biobank.

Methods We performed a phenome-wide association study (PheWAS) to identify disease outcomes associated with SUA genetic risk loci. We then implemented conventional Mendelianrandomisation (MR) analysis to investigate the causal relevance between SUA level and disease outcomes identified from PheWAS. We next applied MR Egger analysis to detect and account for potential pleiotropy, which conventional MR analysis might mistake for causality, and used the HEIDI (heterogeneity in dependent instruments) test to remove cross-phenotype associations that were likely due to genetic linkage.

Results Our PheWAS identified 25 disease groups/outcomes associated with SUA genetic risk loci after multiple testing correction (P<8.57e-05). Our conventional MR analysis implicated a causal role of SUA level in three disease groups: inflammatory polyarthropathies (OR=1.22, 95% CI 1.11 to 1.34), hypertensive disease (OR=1.08, 95% CI 1.03 to 1.14) and disorders of metabolism (OR=1.07, 95% CI 1.01 to 1.14); and four disease outcomes: gout (OR=4.88, 95% CI 3.91 to 6.09), essential hypertension (OR=1.08, 95% CI 1.03 to 1.14), myocardial infarction (OR=1.16, 95% CI 1.03 to 1.30) and coeliac disease (OR=1.41, 95% CI 1.05 to 1.89). After balancing pleiotropic effects in MR Egger analysis, only gout and its encompassing disease group of inflammatory polyarthropathies were considered to be causally associated with SUA level. Our analysis highlighted a locus (*ATXN2/S2HB3*) that may influence SUA level and multiple cardiovascular and autoimmune diseases via pleiotropy.

Conclusions Elevated SUA level is convincing to cause gout and inflammatory polyarthropathies, and might act as a marker for the wider range of diseases with which it associates. Our findings support further investigation on the clinical relevance of SUA level with cardiovascular, metabolic, autoimmune and respiratory diseases.

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INTRODUCTION

Uric acid (UA) is the end product of the exogenous and endogenous purine metabolism, catalysed by the action of xanthine oxidase.[1] Due to the evolved loss of uricase enzyme, humans are unable to convert UA into highly soluble compounds, leaving urate circulating in the blood and resulting in a high basal level of serum uric acid (SUA).[2] The prevalence rate of hyperuricaemia (elevated SUA level >7.0 mg/dL) is in the range of 5%–25% across different countries.[3–5] A progressively rising trend of hyperuricaemia prevalence has been observed worldwide.[5] Concernedly, hyperuricaemia is thought to inflict multiple clinical consequences, which is believed to be causally related to gout and suggestively associated with a number of prevalent health conditions, such as cardiovascular and metabolic diseases.[6–8]

Our recently published umbrella review presented a comprehensive overview of the breadth of disease outcomes related to SUA level by incorporating evidence from multiple sources.[9] A large number of disease outcomes were reported to be associated with SUA level in observational studies, covering a wide range of diseases, including cardiovascular disease, metabolic syndrome, diabetes, cancer and neurological disorders. However, evidence as to whether these associations are actually causal is not yet well developed, given that observational associations are susceptible to a variety of biases, confounding and/or reverse causality. Although results from randomised controlled trials (RCTs) have provided some evidence about the beneficial effects of SUA-lowering therapy on some intermediate traits or biomarkers (eg, blood pressure, endothelial function, serum creatinine), there remains a lack of RCTs focusing on the more important clinical disease endpoints.[10–12] A number of Mendelian randomisation (MR) studies, using the genetic variants influencing SUA level as instruments, provide alternative evidence to distinguish causal from non-causal associations. However, these MR studies examined a limited set of disease outcomes and were not able to detect moderate effect size due to limited power.[13–19] Increasing sample size and the range of outcomes in an enlarged MR study thus offers the prospect of deeper and wider insight into the causal role of SUA.

MR analysis is typically hypothesis-driven based on prior knowledge to specify the outcome to be examined in relation to the exposure of

interest. Traditionally, only one (or a limited number) association between the exposure and one (or a few) predefined outcome(s) is tested in an MR study. Recently, phenome-wide Mendelian randomisation (MR-PheWAS) analysis has been proposed by integrating the phenome-wide association study (PheWAS) and MR method to build a hypothesis-searching approach, which aims to explore potential causal relationships between an exposure (using genetic instruments as proxies) and a range of phenome-wide disease outcomes in a high-throughput manner.[20] This approach is effective in evaluating or replicating the associations reported in observational studies, as well as discovering new relationships and generating new hypotheses on the genetic architecture shared by the related phenotypes. With its wealth of genotypic and phenotypic data collected in very large numbers, the UK Biobank study provides an excellent opportunity to explore the causal role of SUA level across a broad spectrum of disease outcomes. In this study, we performed an MR-PheWAS in UK Biobank database to discover disease outcomes related to genetic variations of SUA level and to investigate if any association is causal.

METHODS

UK Biobank data

The UK Biobank is a large-scale, population-based, prospective cohort that enrolled over 500 000 participants aged 40–69 years. The recruited participants provided a wide range of self-reported baseline information. Blood samples were collected for biochemical tests and genotyping. Their national health records have been linked with the baseline and genotypic data for longitudinal follow-up. Genotypic and phenotypic data used in this study were obtained from UK Biobank under an approved data request application (application ID: 10775).

Genotyping and quality control

Genotyping, quality control and genetic imputation were performed by the UK Biobank team prior to the interim release of genotypic data for 150 000 participants. The procedure of genotyping and quality control is presented in detail at https://biobank.ctsu.ox.ac.uk/crystal/docs/genotyping_qc.pdf. We used the field variables made available by the UK Biobank for quality control to exclude the samples that had high missingness or heterozygosity, outlying short runs of homozygosity, and sex mismatch (see online supplementary table S1). We constrained our analyses to participants who were self-reported British and confirmed to be Caucasians based on the genetic principal component analysis performed by the UK Biobank. The quality control process generated a genotypic data set output with 120 091 individuals included in the current analysis.

Phenotyping and mapping ICD-10 or ICD-9 to phecode

We focused on phenotypes in relation to diagnostic disease outcomes. We analysed two phenotypic data sets (inpatient hospital episode records and cancer registry data) in the UK Biobank using the phecode schema (see online supplementary text for phenotyping and mapping process).[21] The coding for clinical diagnoses in these data sets followed the WHO's International Classification of Diseases (ICD) coding systems, but used different ICD versions (ICD-10 or ICD-9) according to the date of record. We included both ICD-10 and ICD-9 codes to define the case and control groups. Since cancer registry data overlapped with the cancer diagnosis in inpatient hospital records, we pooled the cancer registry data into the hospital episode data as a complement to the cancer diagnosis.

Statistical analysis

The statistical analysis included three main steps: first, we performed a PheWAS to identify disease outcomes that were associated with genetic risk loci of SUA level; second, we performed MR analysis by using both the inverse-variance weighted (IVW) method and MR Egger approach to explore causal relationship for identified PheWAS asscoations[22 23]; and third, we applied HEIDI (heterogeneity in dependent instruments) test to exclude the cross-phenotype associations caused by genetic linkage.[24]

Genetic instruments

We selected 31 SUA-associated single nucleotide polymorphisms (SNPs) as genetic instruments (see online supplementary table S2), which were previously reported to be independently associated with SUA level in genome-wide association studies (GWAS).[25 26] We obtained the SNP effect on SUA level from the largest GWAS performed in European population.[25] The overall proportion of variance (R^2 of SUA level explained by the selected genetic instruments) was estimated to be close to 7.0%.[25]

Phenome-wide association analysis

In phenome-wide analysis, we used 31 SUA-associated SNPs as genetic instruments individually to scan across a wide range of disease outcomes defined by the phecode system.[21] With the PheWAS algorithm,[27] a series of case–control tests was performed: (1) the case group was generated by including patients with the tested phecode; (2) participants were assigned to the control group based on the absence of both the tested phecode and related phecodes (patients who had the parent, child or sibling phecodes of the tested phecode were excluded from the control group)[27]; and (3) to ensure statistical power, analysis was only performed for phecode with no less than 200 cases. This minimum number of cases was suggested based on a simulation of power estimates for PheWAS analysis.[28] We used logistic regression to test the associations between 31 individual genetic instruments (assuming an additive genetic model) and each phecode (number of cases ≥200) after adjusting for multiple covariates, including sex, body mass index (BMI), age, assessment centre and the principal components. Considering many phecodes were not independent, we used the false discovery rate (FDR) method to account for multiple testing.[29]

MR IVW, MR Egger and HEIDI test

We then explored the identified PheWAS associations in three possible scenarios (see online supplementary figure S1): (1) causality: the observed association was causal (through the SUA pathway); (2) pleiotropy: the observed association was due to pleiotropic effect of one causal variant (ie, linked to SUA level and the particular disease outcome through pleiotropy); and (3) genetic linkage: the observed association was caused by the linkage disequilibrium (LD) between two distinct causal variants, with one affecting SUA level and the other affecting the disease outcome.

MR IVW

To explore if there was any causal effect on identified disease outcomes, we performed the conventional MR analysis by pooling the individual effect of each SNP using the IVW method

to estimate the overall causal effect (see online supplementary text).[30]

MR Egger

We then performed MR Egger to attempt to correct for any potential pleiotropic effect in the causal estimates. This approach is applied to balance the pleiotropic effects derived from multiple genetic instruments (see online supplementary text).[23]

HEIDI test

We calculated HEIDI statistics for the SUA genetic loci that were associated with more than one disease outcome. This test was to examine if the cross-phenotype association was due to genetic linkage (see online supplementary text).[24]

Sex stratification analysis

To account for any sex difference, we performed PheWAS and MR analyses in men and women separately. The sex-specific effects of SNPs on SUA level (see online supplementary table S2) were taken from the summary-level GWAS data provided by Köttgen *et al*.[25]

RESULTS

A total of 120 091 UK Biobank participants were included in the analysis, consisting of 56 845 men and 63 246 women with a mean age of 64.86 years in 2016 (SD of 7.95) (see online supplementary table S3). Within phenotypic data sets, we identified 684 324 hospital episodes and 23 174 cancer registration records, which included 7990 unique ICD-10 codes and 1998 unique ICD-9 codes. After mapping diagnostic ICD-10 or ICD-9 codes to phecodes, the phenotypic data consisted of 1807 distinct phecodes. After filtering out disease outcomes with low prevalence (number of cases <200), 568 phecodes (median number of cases=694 (range: 200–39 142)) were included in PheWAS analysis. These 568 phecodes were classified into 17 broadly related disease categories (table 1). We noted that the distribution of phenotypes examined was skewed across the different disease categories (see online supplementary figure S2), in which a large number of disease phenotypes were included in digestive, circulatory, endocrine and metabolic systems, but some disease categories, for example congenital anomalies, were not well represented in the study population.

Phenome-wide association analysis

The PheWAS analysis performed 17 608 case–control tests, leading to an adjusted significance threshold of P<8.57e-05 corresponding to an FDR of q<0.05 to account for the multiple testing. A total of 27 pairs of genotype–phenotype associations passed the significance threshold of FDR correction (P<8.57e-05) in the overall PheWAS analysis with adjustment for covariates (table 2). Results of PheWAS without adjustment for BMI are shown in online supplementary table S4. The sex-stratified PheWAS analysis identified 10 pairs of genotype–phenotype association in men and 10 pairs of genotype–phenotype association in women (see online supplementary table S5). When compared with the overall PheWAS analysis, five new pairs of association were identified from the sex-stratified PheWAS analysis (see online supplementary table S5).

These identified genotype–phenotype associations were distributed across 15 SUA genetic loci, of which 5 loci were associated with more than one disease outcome: rs653178 in *ATXN2/SH2B3* locus (number of disease outcomes: $n_{outcomes}$ = 10), rs1165151 in *SLC17A3* locus ($n_{outcomes}$ = 3), rs1260326 in *GCKR* locus ($n_{outcomes}$ = 3), rs2231142 in *ABCG2* locus ($n_{outcomes}$ = 4) and rs2079742 in *BCAS3* locus ($n_{outcomes}$ = 2). Of note, six disease outcomes shared genetic associations with SUA level at more than one locus: gout (number of loci: n_{loci} = 3), inflammatory polyarthropathies (n_{loci} = 2), disorders of iron metabolism (n_{loci} = 2), coeliac disease (n_{loci} = 2), hypertensive disease (n_{loci} = 2) and essential hypertension (n_{loci} = 2).

In summary, the PheWAS analyses identified 25 unique disease groups/outcomes (corresponding to 25 unique phecodes) that shared genetic risk loci with SUA level, which included 9 disease groups (inflammatory polyarthropathies, hypertensive disease, circulatory disease, disorders of metabolism, disorders of thyroid, other diseases of respiratory system, disorder of skin and subcutaneous tissue, benign neoplasm of digestive system, and complications of labour and delivery) and 16 specific disease

Table 1 The number of phenotypes and the number of cases in each disease category

		Cases (n)			
Disease categories	**Phenotypes (n)**	**Minimum**	**Median**	**Mean**	**Maximum**
Circulatory system	61	221	665	2937	39142
Congenital anomalies	6	206	265	302	522
Dermatological diseases	24	201	706	2736	32 738
Diseases in sense organs	34	201	425	1216	11306
Digestive diseases	73	201	949	2176	23129
Neoplasms	59	203	763	1916	30101
Infectious diseases	16	205	787	975	3192
Endocrine and metabolic diseases	25	229	492	2304	13592
Haematopoietic diseases	10	205	1187	1600	3669
Neurological diseases	21	229	452	1282	11828
Respiratory diseases	38	219	712	1713	19238
Mental disorders	18	205	673	1926	8942
Genitourinary diseases	77	200	666	1606	29859
Pregnancy complications	11	227	360	707	2531
Musculoskeletal diseases	44	263	1076	2482	21822
Clinical symptoms	14	267	1237	2570	12287
Injuries and poisonings	37	211	589	911	4842

Table 2 Genotype–phenotype associations identified from PheWAS after correcting multiple testing by FDR (P<8.57e-05)

Phecode	Description	SNP_risk allele*	Allele frequency	Total (n)	Cases (n)	OR (95% CI)	P†
274.1	Gout	rs2231142_T	0.11	119555	1003	1.89 (1.69 to 2.12)	5.41e-28
275.1	Disorders of iron metabolism	rs1165151_G	0.45	119063	205	3.56 (2.78 to 4.56)	1.41e-23
244.4	Hypothyroidism	rs653178_C	0.48	118821	4146	1.21 (1.16 to 1.27)	3.90e-17
246	Disorders of thyroid	rs653178_C	0.48	119601	4926	1.18 (1.14 to 1.23)	8.82e-16
274.1	Gout	rs12498742_A	0.23	118960	1002	1.54 (1.37 to 1.74)	7.94e-13
275.1	Disorders of iron metabolism	rs742132_A	0.29	119271	205	2.80 (2.10 to 3.74)	3.13e-12
401	Hypertensive disease	rs653178_C	0.48	119762	23634	1.06 (1.04 to 1.09)	1.68e-08
401.1	Essential hypertension	rs653178_C	0.48	119688	23560	1.06 (1.04 to 1.09)	2.00e-08
411.4	Coronary atherosclerosis	rs653178_C	0.48	119460	9526	1.09 (1.05 to 1.12)	1.27e-07
411	Ischaemic heart disease	rs653178_C	0.48	119401	9467	1.09 (1.05 to 1.12)	1.33e-07
211	Benign neoplasm of digestive system	rs11264341_C	0.43	117030	1504	0.83 (0.77 to 0.89)	2.41e-07
274.1	Gout	rs1260326_T	0.39	119555	1003	1.26 (1.15 to 1.38)	3.86e-07
459.9	Circulatory disease	rs653178_C	0.48	119677	39142	1.05 (1.03 to 1.06)	2.24e-06
411.2	Myocardial infarction	rs653178_C	0.48	113559	3625	1.12 (1.07 to 1.18)	2.80e-06
557.1	Coeliac disease	rs1165151_G	0.45	99783	549	1.33 (1.18 to 1.51)	4.30e-06
557.1	Coeliac disease	rs653178_C	0.48	99965	550	1.31 (1.16 to 1.48)	9.28e-06
427.2	Atrial fibrillation and flutter	rs6598541_A	0.35	113261	4333	1.11 (1.06 to 1.16)	9.92e-06
960	Poisoning by antibiotics	rs1165151_G	0.45	112343	1027	0.82 (0.75 to 0.90)	1.22e-05
535	Gastritis and duodenitis	rs478607_G	0.15	115386	5233	1.12 (1.07 to 1.19)	1.34e-05
411.3	Angina pectoris	rs653178_C	0.48	114967	5033	1.09 (1.05 to 1.14)	3.01e-05
669	Complications of labour and delivery	rs729761_G	0.28	113240	2376	1.17 (1.09 to 1.26)	3.78e-05
272.11	Hypercholesterolaemia	rs1260326_T	0.39	118921	10201	1.07 (1.03 to 1.10)	3.82e-05
366	Cataract	rs6770152_G	0.43	116218	4567	1.09 (1.05 to 1.14)	4.14e-05
471	Nasal polyps	rs10821905_A	0.17	112745	983	1.26 (1.13 to 1.40)	4.61e-05
454.1	Varicose veins of lower extremity	rs2231142_T	0.11	111390	3204	0.84 (0.78 to 0.92)	5.79e-05
401	Hypertensive disease	rs2079742_T	0.13	115659	22832	1.07 (1.03 to 1.10)	7.00e-05
401.1	Essential hypertension	rs2079742_T	0.13	115588	22761	1.07 (1.03 to 1.10)	7.02e-05

*Effect allele was harmonised to be the SUA-raising allele defined by Köttgen *et al*.[25]
†Significance threshold of P<8.57e-05 corresponds to an FDR of q<0.05 after correcting the multiple testing.
FDR, false discovery rate; PheWAS, phenome-wide association study; SUA, serum uric acid.

outcomes (gout, essential hypertension, angina pectoris, myocardial infraction, coronary atherosclerosis, ischaemic heart disease, atrial fibrillation and flutter, varicose veins of lower extremity, hypercholesterolaemia, disorders of iron metabolism, coeliac disease, hypothyroidism, gastritis and duodenitis, poisoning by antibiotics, cataract, and nasal polyps). The mappings of ICD codes to these 25 phecodes and their hierarchical relationships are shown in online supplementary table S6.

MR IVW, MR Egger and HEIDI test

We then performed MR analysis using the IVW method to explore if there was any causal link between SUA level and the 25 disease groups/outcomes identified from PheWAS analysis. The MR IVW analysis suggested a potential causal link for 7 out of 25 disease groups/outcomes. The corresponding effect estimate on each disease outcome is presented in table 3. It was indicated that genetically determined higher SUA level was potentially causally linked with an increased risk of three disease groups, including inflammatory polyarthropathies (OR=1.22, 95% CI 1.11 to 1.34, P=1.10e-04), hypertensive disease (OR=1.08, 95% CI 1.03 to 1.14, P=0.004) and disorders of metabolism (OR=1.07, 95% CI 1.01 to 1.14, P=0.03), and of four specific disease outcomes, including gout (OR=4.88, 95% CI 3.91 to 6.09, P=3.55e-15), essential hypertension (OR=1.08, 95% CI 1.03 to 1.14, P=0.005), myocardial infarction (OR=1.16, 95% CI 1.03 to 1.30, P=0.015) and coeliac disease (OR=1.41, 95% CI 1.05 to 1.89, P=0.02).

To explore and correct for any possible pleiotropic effect of multiple instruments, we then conducted the MR Egger analysis (table 3). After balancing out the potential pleiotropic effects, the putative causal link of SUA level with gout (OR=4.58, 95% CI 2.72 to 7.72, P_{effect}= 1.76e-06) and its umbrella disease group, inflammatory polyarthropathies (OR=1.15, 95% CI 1.01 to 1.31, P_{effect}=0.03), remained statistically significant and there was no indication of unbalanced pleiotropy ($P_{pleiotropy}$=0.73 and $P_{pleiotropy}$=0.23, respectively). The putative causal effect of SUA level on the other five disease groups/outcomes was not statistically significant in the MR Egger model. The causal effects of each individual SNPs on these seven disease groups/outcomes are shown in online supplementary figures S3–S9. Unbalanced pleiotropy was observed for essential hypertension ($P_{pleiotropy}$ =0.001) and its umbrella disease group, hypertensive disease ($P_{pleiotropy}$=0.001). For myocardial infarction, coeliac disease and disorders of metabolism, the putative causal effect was not statistically significant in the MR Egger model (P_{effect}=0.75, P_{effect} =0.41 and P_{effect}=0.80, respectively), although there was no evidence of unbalanced pleiotropy ($P_{pleiotropy}$=0.13, $P_{pleiotropy}$ =0.75 and $P_{pleiotropy}$=0.18, respectively). The results of the sex-stratified MR IVW are presented in online supplementary table S7.

Finally, to distinguish the genotype–phenotype association of pleiotropy from LD, the HEIDI test was performed for the five genetic loci (rs653178 in *ATXN2/SH2B3* locus, rs1165151

Table 3 PheWAS associations assessed by conventional MR IVW and MR Egger analysis

	MR IVW			MR Egger			
Disease outcomes	**OR (95% CI)**	**P effect**	**Power***	**OR (95% CI)**	**P effect**	**P pleiotropy**	**Power***
Gout	4.88 (3.91 to 6.09)	3.55e-15	1.00	4.58 (2.72 to 7.72)	1.76e-06	0.73	1.00
Inflammatory polyarthropathies†	1.22 (1.11 to 1.34)	1.10e-04	0.99	1.15 (1.01 to 1.31)	0.03	0.23	0.83
Essential hypertension	1.08 (1.03 to 1.14)	5.07e-03	0.82	0.93 (0.83 to 1.05)	0.23	1.13e-03	0.73
Hypertensive disease	1.08 (1.03 to 1.14)	4.23e-03	0.82	0.93 (0.83 to 1.05)	0.24	1.19e-03	0.73
Myocardial infarction	1.16 (1.03 to 1.30)	0.02	0.70	1.03 (0.84 to 1.27)	0.75	0.13	0.08
Coeliac disease	1.41 (1.05 to 1.89)	0.02	0.72	1.31 (0.68 to 2.54)	0.41	0.75	0.48
Disorders of metabolism†	1.07 (1.01 to 1.14)	0.03	0.52	1.01 (0.91 to 1.14)	0.80	0.18	0.06
Coronary atherosclerosis	1.07 (0.99 to 1.15)	0.08	0.41	0.99 (0.85 to 1.17)	0.95	0.20	0.06
Ischaemic heart disease	1.07 (0.99 to 1.15)	0.09	0.41	0.99 (0.85 to 1.16)	0.91	0.20	0.06
Angina pectoris	1.04 (0.94 to 1.15)	0.41	0.11	0.95 (0.80 to 1.12)	0.51	0.11	0.15
Atrial fibrillation and flutter	1.01 (0.91 to 1.12)	0.87	0.05	0.90 (0.75 to 1.08)	0.23	0.07	0.41
Circulatory disease	1.04 (1.00 to 1.09)	0.08	0.40	0.97 (0.89 to 1.07)	0.57	0.05	0.26
Varicose veins of lower extremity	0.86 (0.72 to 1.02)	0.09	0.55	0.86 (0.67 to 1.10)	0.24	0.97	0.55
Disorders of iron metabolism	1.19 (0.74 to 1.90)	0.45	0.11	0.79 (0.15 to 4.07)	0.77	0.47	0.12
Hypercholesterolaemia	1.14 (0.96 to 1.36)	0.12	0.94	1.18 (0.88 to 1.58)	0.27	0.78	0.99
Hypothyroidism	1.10 (0.99 to 1.23)	0.07	0.39	0.99 (0.75 to 1.32)	0.97	0.30	0.05
Disorders of thyroid	1.08 (0.98 to 1.20)	0.10	0.31	1.01 (0.79 to 1.29)	0.94	0.41	0.05
Benign neoplasm of digestive system	0.93 (0.78 to 1.10)	0.36	0.11	0.90 (0.64 to 1.26)	0.52	0.79	0.18
Gastritis and duodenitis	0.97 (0.88 to 1.07)	0.53	0.09	0.95 (0.80 to 1.13)	0.55	0.70	0.16
Nasal polyps	1.08 (0.88 to 1.34)	0.45	0.10	1.09 (0.73 to 1.60)	0.67	0.98	0.12
Cataract	0.99 (0.90 to 1.09)	0.85	0.05	0.91 (0.75 to 1.10)	0.34	0.23	0.36
Poisoning by antibiotics	0.85 (0.70 to 1.04)	0.14	0.25	1.00 (0.68 to 1.48)	1.00	0.28	0.05
Complications of labour and delivery†	0.89 (0.76 to 1.03)	0.12	0.30	0.78 (0.59 to 1.02)	0.08	0.20	0.83
Other diseases of respiratory system†	1.11 (0.94 to 1.31)	0.19	0.22	1.16 (0.92 to 1.46)	0.22	0.64	0.42
Disorder of skin and subcutaneous tissue†	0.99 (0.93 to 1.06)	0.77	0.06	0.98 (0.89 to 1.09)	0.75	0.85	0.09

*The statistical power of MR analyses was calculated by using the non-centrality parameter-based approach[65]; the overall proportion of variance (R^2) of serum uric acid level explained by the genetic instruments was estimated to be 7.0%.[25]
†Disease outcomes identified from sex-stratified PheWAS analysis.
IVW, inverse-variance weighted; MR, Mendelian randomisation; PheWAS, phenome-wide association study.

in *SLC17A3* locus, rs1260326 in *GCKR* locus, rs2231142 in *ABCG2* locus and rs2079742 in *BCAS3* locus) that were associated with multiple disease outcomes in the PheWAS analysis (see online supplementary figures S10–S14). Based on the HEIDI test, we identified 14 disease outcomes that were associated with the SUA genetic risk loci due to pleiotropy (with P_{HEIDI} >0.05). The strongest pleiotropic locus was the *ATXN2/SH2B3*, where three SNPs (rs653178, rs4766578 and rs3184504) in near-complete LD (r^2=0.99) were tagged as the lead SNPs associated with 10 disease groups/outcomes as a cluster of cardiovascular diseases and autoimmune disorders (see online supplementary figure S10). Other potential pleiotropic effects included the associations of *BCAS3* locus (rs2079742) with essential hypertension (P_{HEIDI}=0.10) and hypertensive disease (P_{HEIDI}=0.09) (see online supplementary figure S11), the associations of *ABCG2* locus (rs2231142) with varicose veins of lower extremity (P_{HEIDI}=0.32) (see online supplementary figure S12), and the association of *SLC17A3* locus (rs1165151) with poisoning by antibiotics (P_{HEIDI}=0.26) (see online supplementary figure S13).

Our analysis rejected the null hypothesis of a pleiotropic model for the shared genetic association between SUA level and disorders of iron metabolism at the *SLC17A3* locus (rs1165151) (P_{HEIDI}=5.54e-28); we identified a different causal variant (rs17342717 in *SLC17A1*) that was in LD with the SNP rs1165151 (r^2=0.24) and strongly associated with the disorders of iron metabolism (P=1.69e-129) (see online supplementary figure S13). Similarly, for the associations between the *SLC17A3* locus (rs1165151) and coeliac disease (P_{HEIDI}=6.51e-16) (see online supplementary figure S13), and the *GCKR* locus (rs1260326) and hypercholesterolaemia (P_{HEIDI}=3.27e-11) (see online supplementary figure S14), the pattern of shared regional genetic association was more consistent with a genetic linkage model, and the SNP with the smallest P value was tagged as an index of the distinct causal variant affecting the examined disease outcome.

DISCUSSION

In PheWAS analysis by using SUA-associated SNPs as genetic instruments, we replicated the findings of the largest GWAS performed by Köttgen and the findings of the most recent candidate gene-based association study conducted in UK Biobank, which indicated that two SUA-related SNPs (rs12498742 in *SLC2A9* locus and rs2231142 in *ABCG2* locus) are significantly associated with gout at GWAS P value threshold (P<5.0e-08).[25 31] We conducted a conventional MR analysis (using the IVW method) and an MR Egger analysis, which accounts for potential pleiotropic effects, to investigate potential causal links with SUA level. These both confirmed potential causal effects of SUA level on gout and inflammatory polyarthropathies. The latter category represents the disease group term that includes gout, and thus this finding may just reflect the causal role of SUA in gout. However, this study cannot exclude a causal association between SUA and other inflammatory polyarthropathies, and this may be worth further study. Given that many comorbidities

are commonly reported in patients with gout, it is of interest to consider the evidence for SUA sharing genetic risk loci with some of these diseases, such as cardiovascular/metabolic diseases and autoimmune disorders, and the evidence for a possible causal role for SUA in these conditions.

Overall, we identified 32 pairs of genotype–phenotype associations, which covered a wide range of phenotypic categories including endocrine/metabolic diseases, cardiovascular diseases and autoimmune disorders. Our PheWAS analysis replicated 14 pairs of previously known genotype–phenotype (or closely related phenotypic groups) associations reported in the GWAS Catalog (see online supplementary table S2 and table 2). For example, rs653178 (*ATXN2/SH2B3* locus) was previously reported to be associated with diastolic blood pressure,[32] myocardial infarction,[33] peripheral artery disease,[34] coeliac disease[35] and serum thyroid peroxidase antibody levels.[36] In our PheWAS, this SNP was statistically significantly associated with the same phenotypes (ie, coeliac disease, myocardial infarction) or similar phenotypic groups (ie, hypertension, circulatory and heart diseases, hypothyroidism and other disorders of thyroid). We also identified 18 novel genotype–phenotype associations (at the PheWAS threshold of P<8.57e-05), of which the association between rs1165151 (*SLC17A3* locus) and disorders of iron metabolism had the smallest P value (P=1.23e-19).

We performed conventional MR analysis, using the IVW method, to investigate whether there was a potential causal link between SUA level and the 25 unique disease groups/outcomes identified from PheWAS. The results of MR IVW analysis suggested a potential causal effect of SUA level on three disease groups, including inflammatory polyarthropathies (as noted above), hypertensive disease and disorders of metabolism, and four specific individual disease outcomes, including gout (as noted above), essential hypertension, myocardial infarction and coeliac disease. When adopting the advanced MR Egger analysis to account for potential pleiotropic effects, it is indicated that, except for gout and inflammatory polyarthropathies, all the other putative causal associations suggested by MR IVW analysis were probably inflated by the presence of pleiotropy. However, although the MR Egger analysis is more robust in dealing with pleiotropy, this method is not infallible.[37] Intuitively, the genetic instrument with larger effect on SUA level is expected to have a larger effect on disease outcome and would exert stronger influence in the MR Egger regression model. With indepth examination of the individual SNP effects on SUA level against the SNP effects on disease outcomes (see online supplementary figures S5–S8), we found that the outlying variant (rs12498742 in *SLC2A9*) that had the strongest association with SUA level showed a negative (null) effect on essential hypertension and hypertensive disease, which reversed the sign of the overall putative causal effect and led to a rejection of the intercept test. Given the influence of the outlying variant, the unbalanced pleiotropy and relatively moderate statistical power (power=0.73), we would interpret that unbalanced pleiotropy between SUA level and hypertension is an issue for their causal inference in MR Egger analysis.

Previous observational studies have reported sex difference in the association between SUA level and the development of cardiovascular diseases,[38–42] but few studies have addressed the sex difference by using MR approach to keep out the influence of environmental confounders. Our study identified a few more cardiovascular diseases (eg, coronary atherosclerosis, ischaemic heart disease) that were potentially causally linked with the genetic variation of SUA level in women, but not in men. These MR findings were concordant with results from observational studies, which indicated that the relationship between SUA level and cardiovascular disease was particularly strong in women, especially for heart disease.[41 43 44] Although these putative causal associations specific to women were not verified by MR Egger, this may be due to the decreased statistical power of MR Egger (and a higher risk of type 2 error). The biological mechanism that can lead the association of SUA level with cardiovascular disease to be more pronounced in women than in men remains a matter for further investigation.

We also found that several PheWAS associations were likely driven by LD. For instance, the outstanding PheWAS association between disorders of iron metabolism and the SNP rs1165151 in *SLC17A3* locus was not consistent with a pleiotropic model, and further examination found the SUA-associated SNP rs1165151 was located in LD (r^2=0.24), with the rs17342717 variant in *SLC17A1* locus, which was strongly associated with disorders of iron metabolism (P=1.69e-129). This SNP (rs17342717) is also associated with red blood cell traits and serum iron levels in previous GWAS.[45 46] We suggest that the implications of these findings have wider relevance for PheWAS studies. Typically, associations of a single SNP with multiple phenotypes were claimed to be due to pleiotropy in previous PheWAS.[47 48] However, as PheWAS focused on single variant without considering the correlations between SNPs, we would suggest that an additional examination of LD is necessary when we identify pleiotropic links.

In contrast, the pattern of shared regional genetic associations of SUA level with multiple disease outcomes at *ATXN2/S2HB3* locus was more consistent with a pleiotropic model, where we interpreted this locus influenced a cluster of cardiovascular diseases and autoimmune disorders. However within the *ATXN2/S2HB3* locus, there are three leading SNPs (rs653178, rs4766578 and rs3184504) in high LD (r^2=0.99). In this case, we were unable to provide an indication of whether the observed associations are due to pleiotropy or genetic linkage, as it was difficult to infer the causal variant. Although SNP rs653178 was reported as the lead variant influencing SUA level at this locus in GWAS, the potential biological mechanism underlying this effect is unclear.[25] Furthermore, although the implication of the rs653178 on the regulation of blood pressure, cardiovascular diseases and coeliac disease has been suggested by a few GWAS,[32–35] a clear biological explanation for this role could not be demonstrated. Evidence from the functional follow-up of the *S2HB3* gene indicated that rs3184504 may be the causal variant, as the *S2HB3* gene encodes one of the S2HB family proteins, which have a diverse physiological roles on haematopoiesis, immune response and signalling, and variation in rs3184504 may introduce a new phosphorylation site affecting the function of the S2HB protein.[49 50] We believe that further uncovering of the biological functions of this pleiotropic locus (eg, gene function follow-up, expression quantitative trait loci analysis) might be helpful to understand the complex underlying relationship of SUA level with cardiovascular and autoimmune diseases.

The sex-stratified MR IVW analysis identified that unspecified diseases in respiratory system were potentially causally linked with SUA level in women (with the MR Egger analysis showing a consistent causal effect). This finding is consistent with recently published experimental studies, which demonstrated that human airway epithelial cells and lung tissue expressed a functional UA production/secretion system and UA was crucial in mediating the development of allergic airway diseases and regulating the antigen-specific T cell proliferation.[51–54] It was also speculated that fine, inhaled particulate matter can induce increased UA production in the human airway, which may contribute to

allergic sensitisation and asthma pathogenesis.[55] Evidence from other epidemiological studies suggested that high SUA level was associated with low lung function and high risk of respiratory symptoms and chronic obstructive pulmonary disease, but the direct causal relationship has not been established.[56–58] Further investigation may be worth to explore the clinical relevance of SUA level in lung health and respiratory diseases.

Key strengths of our study included its potential to make novel discoveries in genotype–phenotype associations and to identify novel cross-phenotype associations, possibly reflecting common aetiology or causal mechanisms. Unlike the genome, for which genetic structure can be measured by reliable biological techniques, the definition of phenome varies across studies. Current published PheWAS have been limited primarily to billing ICD-9-clinical modification (CM) to phecode system, and the method for aggregating ICD-9-CM codes into phecodes has proven to be valuable in previous PheWAS studies.[21 59] Our work broadened the utility of phecode system and illustrated the process of adopting phecode system in the updated ICD-10 version to define the phenome framework. Our mapping process revealed some potential shortcomings of the current phecode system (eg, the ICD-10 codes involving the personal or family history were missing elements in the phecode system), which should be improved as a future undertaking. Recent methodological applications (eg, tree-structured phenotypic model (TreeWAS)) can be applied in future PheWAS analyses.[60] As we were preparing the manuscript for submission, a web resource within UK Biobank, the GeneATLAS, was released in the bioRxiv (prior to peer review).[61] We checked our PheWAS findings in this database, but only 10 of the 31 SUA-related SNPs were included in their database (and associations with some disease outcomes were replicated for these SNPs).[61] We focused on the causal relationships between SUA level and binary disease outcomes in MR analyses, and these findings were complementary to MR estimates of urate archived in the MR-Base database (http://eve.mrbase.org/), which mainly focused on quantitative traits.

On the other hand, our analysis was limited to phenotypes with no less than 200 cases; therefore, diseases with relatively low prevalence were not analysed. As the UK Biobank grows, we expect to perform PheWAS and MR analyses for more phenotypes, with the priority given to the ones of which the relationships with SUA level are much controversial, such as dementia.[62 63] Furthermore for some analysed phenotypes, our PheWAS analysis may still have low power to detect small effect size. The use of the interim release of UK Biobank data and focusing on a very homogeneous population (self-reported British confirmed by principal component analysis (PCA)) limited the power of this study. Additionally, we did not analyse the self-reported UK Biobank data to avoid information bias, but this may have impacted on the comprehensiveness of PheWAS and have reduced the precision of MR estimates. To improve this limitation, we performed a sensitivity analysis for gout by comparing the MR estimates for hospital-diagnosed gout, self-reported gout and hospital-diagnosed/self-reported gout (see online supplementary table S8). The MR estimates were consistently statistically significant in any of the cases but with differences in their effect sizes. These differences might be due to the fact that gout cases ascertained from hospital discharge coding may be unrepresentative of gout, given hospitalised gout is more likely to be complicated by comorbidities, as reported by Robinson *et al*.[64] While making efforts to dissect the PheWAS associations with different models, given the complexity of human genetic structure, these models are not mutually exclusive and each model has its own methodological limitations, thus strong conclusions are not always possible. Therefore, the realistic goal for the present study was to assess different lines of evidence (ie, causality, pleiotropy or genetic linkage) in order to characterise the identified PheWAS associations in relation to SUA level. It would be beneficial to assess whether measured SUA level, rather than its genetic proxy, is also associated with the observed disease outcomes, but data on the SUA biomarker are not yet available in UK Biobank.

Overall, this PheWAS analysis demonstrated that SUA level shares genetic risk loci with multiple disease outcomes, particularly cardiovascular/metabolic diseases and autoimmune disorders. These findings provide rationale for further investigation of whether these associations are causal. Our study indicated a putative causal effect of SUA level on three disease groups (inflammatory polyarthropathies, hypertensive disease and disorders of metabolism) and four specific disease outcomes (gout, essential hypertension, coeliac disease and myocardial infarction); when balancing out the pleiotropy, a robust conclusion about causality was made for gout and its encompassing disease group, inflammatory polyarthropathies. Unbalanced pleiotropy was identified as an issue for the causal inference on the association between SUA level and hypertension. Other potential causal relevance of SUA level with respiratory diseases is also worthy of further investigation. When interpreting the PheWAS associations from a view of pleiotropy, our analysis highlighted a key pleiotropic locus that influenced SUA level and multiple cardiovascular and autoimmune diseases. A further functional annotation of this locus might be helpful to understand the biological pathways that contribute to the phenotypic associations between SUA level and cardiovascular diseases (including hypertension).

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REFERENCES

1 Maiuolo J, Oppedisano F, Gratteri S, *et al*. Regulation of uric acid metabolism and excretion. *Int J Cardiol* 2016;213:8–14.
2 Oda M, Satta Y, Takenaka O, *et al*. Loss of urate oxidase activity in hominoids and its evolutionary implications. *Mol Biol Evol* 2002;19:640–53.
3 Zhu Y, Pandya BJ, Choi HK. Prevalence of gout and hyperuricemia in the US general population: the National Health and Nutrition Examination Survey 2007-2008. *Arthritis Rheum* 2011;63:3136–41.
4 Liu R, Han C, Wu D, *et al*. Prevalence of Hyperuricemia and Gout in Mainland China from 2000 to 2014: A Systematic Review and Meta-Analysis. *Biomed Res Int* 2015;2015:1–12.
5 Emma Smith LM. Global Prevalence of Hyperuricemia: A Systematic Review of Population-Based Epidemiological Studies. *Arthritis Rheumatol* 2015;67(suppl 10().
6 Feig DI, Kang D-H, Johnson RJ. Uric Acid and Cardiovascular Risk. *N Engl J Med Overseas Ed* 2008;359:1811–21.
7 Culleton BF, Larson MG, Kannel WB, *et al*. Serum uric acid and risk for cardiovascular disease and death: the Framingham Heart Study. *Ann Intern Med* 1999;131:7–13.
8 Nejatinamini S, Ataie-Jafari A, Qorbani M, *et al*. Association between serum uric acid level and metabolic syndrome components. *J Diabetes Metab Disord* 2015;14:70.
9 Li X, Meng X, Timofeeva M, *et al*. Serum uric acid levels and multiple health outcomes: umbrella review of evidence from observational studies, randomised controlled trials, and Mendelian randomisation studies. *BMJ* 2017;357:j2376.
10 Agarwal V, Hans N, Messerli FH. Effect of allopurinol on blood pressure: a systematic review and meta-analysis. *J Clin Hypertens* 2013;15:435–42.
11 Kanbay M, Siriopol D, Nistor I, *et al*. Effects of allopurinol on endothelial dysfunction: a meta-analysis. *Am J Nephrol* 2014;39:348–56.
12 Zhang YF, He F, Ding HH, *et al*. Effect of uric-acid-lowering therapy on progression of chronic kidney disease: a meta-analysis. *J Huazhong Univ Sci Technolog Med Sci* 2014;34:476–81.
13 Pfister R, Barnes D, Luben R, *et al*. No evidence for a causal link between uric acid and type 2 diabetes: a Mendelian randomisation approach. *Diabetologia* 2011;54:2561–9.
14 Kleber ME, Delgado G, Grammer TB, *et al*. Uric Acid and Cardiovascular Events: A Mendelian Randomization Study. *J Am Soc Nephrol* 2015;26:2831–8.
15 Sluijs I, Holmes MV, van der Schouw YT, *et al*. A Mendelian randomization study of circulating uric acid and type 2 diabetes. *Diabetes* 2015;64:3028–36.
16 White J, Sofat R, Hemani G, *et al*. Plasma urate concentration and risk of coronary heart disease: a Mendelian randomisation analysis. *Lancet Diabetes Endocrinol* 2016;4:327–36.
17 Keenan T, Zhao W, Rasheed A, *et al*. Causal assessment of serum urate levels in cardiometabolic diseases through a mendelian randomization study. *J Am Coll Cardiol* 2016;67:407–16.
18 Hughes K, Flynn T, de Zoysa J, *et al*. Mendelian randomization analysis associates increased serum urate, due to genetic variation in uric acid transporters, with improved renal function. *Kidney Int* 2014;85:344–51.
19 Palmer TM, Nordestgaard BG, Benn M, *et al*. Association of plasma uric acid with ischaemic heart disease and blood pressure: mendelian randomisation analysis of two large cohorts. *BMJ* 2013;347:f4262.
20 Millard LA, Davies NM, Timpson NJ, *et al*. MR-PheWAS: hypothesis prioritization among potential causal effects of body mass index on many outcomes, using Mendelian randomization. *Sci Rep* 2015;5:16645.
21 Denny JC, Bastarache L, Ritchie MD, *et al*. Systematic comparison of phenome-wide association study of electronic medical record data and genome-wide association study data. *Nat Biotechnol* 2013;31:1102–11.
22 Burgess S, Scott RA, Timpson NJ, *et al*. Using published data in Mendelian randomization: a blueprint for efficient identification of causal risk factors. *Eur J Epidemiol* 2015;30:543–52.
23 Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol* 2015;44:512–25.
24 Zhu Z, Zhang F, Hu H, *et al*. Integration of summary data from GWAS and eQTL studies predicts complex trait gene targets. *Nat Genet* 2016;48:481–7.
25 Köttgen A, Albrecht E, Teumer A, *et al*. Genome-wide association analyses identify 18 new loci associated with serum urate concentrations. *Nat Genet* 2013;45:145–54.
26 Kolz M, Johnson T, Sanna S, *et al*. Meta-analysis of 28,141 individuals identifies common variants within five new loci that influence uric acid concentrations. *PLoS Genet* 2009;5:e1000504.
27 Carroll RJ, Bastarache L, Denny JC. R PheWAS: data analysis and plotting tools for phenome-wide association studies in the R environment. *Bioinformatics* 2014;30:2375–6.
28 Verma A, Verma SS, Pendergrass SA, *et al*. A simulation study investigating power estimates in Phenome-Wide Association Studies. *bioRxiv* 2017.
29 Benjamini Yoav HY. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society* 1995;72:405–16.
30 Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. *Genet Epidemiol* 2013;37:658–65.
31 Cadzow M, Merriman TR, Dalbeth N. Performance of gout definitions for genetic epidemiological studies: analysis of UK Biobank. *Arthritis Res Ther* 2017;19:181.
32 Newton-Cheh C, Johnson T, Gateva V, *et al*. Genome-wide association study identifies eight loci associated with blood pressure. *Nat Genet* 2009;41:666–76.
33 Nikpay M, Goel A, Won HH, *et al*. A comprehensive 1,000 Genomes-based genome-wide association meta-analysis of coronary artery disease. *Nat Genet* 2015;47:1121–30.
34 Kullo IJ, Shameer K, Jouni H, *et al*. The ATXN2-SH2B3 locus is associated with peripheral arterial disease: an electronic medical record-based genome-wide association study. *Front Genet* 2014;5:166.
35 Dubois PC, Trynka G, Franke L, *et al*. Multiple common variants for celiac disease influencing immune gene expression. *Nat Genet* 2010;42:295–302.
36 Medici M, Porcu E, Pistis G, *et al*. Identification of novel genetic Loci associated with thyroid peroxidase antibodies and clinical thyroid disease. *PLoS Genet* 2014;10:e1004123.
37 Burgess S, Thompson SG. Interpreting findings from Mendelian randomization using the MR-Egger method. *Eur J Epidemiol* 2017;32.
38 Yoshitomi R, Fukui A, Nakayama M, *et al*. Sex differences in the association between serum uric acid levels and cardiac hypertrophy in patients with chronic kidney disease. *Hypertens Res* 2014;37:246–52.
39 Li YH, Lin GM. Sex differences in the association between serum uric acid and cardiac mortality in Asian patients with established coronary artery disease: A revisit of the ET-CHD registry in Taiwan, 1997-2003. *J Cardiol* 2016;68:461.
40 Rodrigues SL, Baldo MP, Capingana P, *et al*. Gender distribution of serum uric acid and cardiovascular risk factors: population based study. *Arq Bras Cardiol* 2012;98:13–21.
41 Freedman DS, Williamson DF, Gunter EW, *et al*. Relation of serum uric acid to mortality and ischemic heart disease. The NHANES I Epidemiologic Follow-up Study. *Am J Epidemiol* 1995;141:637–44.
42 Kurata A, Shigematsu Y, Higaki J. Sex-related differences in relations of uric acid to left ventricular hypertrophy and remodeling in Japanese hypertensive patients. *Hypertens Res* 2005;28:133–9.
43 Feig DI, Kang DH, Johnson RJ. Uric acid and cardiovascular risk. *N Engl J Med* 2008;359:1811–21.
44 Kawabe M, Sato A, Hoshi T, *et al*. Gender differences in the association between serum uric acid and prognosis in patients with acute coronary syndrome. *J Cardiol* 2016;67:170–6.
45. Kullo IJ, Ding K, Jouni H, *et al*. A genome-wide association study of red blood cell traits using the electronic medical record. *PLoS One* 2010;5:e13011.
46 Pichler I, Minelli C, Sanna S, *et al*. Identification of a common variant in the TFR2 gene implicated in the physiological regulation of serum iron levels. *Hum Mol Genet* 2011;20:1232–40.
47 Pendergrass SA, Brown-Gentry K, Dudek S, *et al*. Phenome-wide association study (PheWAS) for detection of pleiotropy within the Population Architecture using Genomics and Epidemiology (PAGE) Network. *PLoS Genet* 2013;9:e1003087.
48 Hall MA, Verma A, Brown-Gentry KD, *et al*. Detection of pleiotropy through a Phenome-wide association study (PheWAS) of epidemiologic data as part of the Environmental Architecture for Genes Linked to Environment (EAGLE) study. *PLoS Genet* 2014;10:e1004678.
49 Devallière J, Charreau B. The adaptor Lnk (SH2B3): an emerging regulator in vascular cells and a link between immune and inflammatory signaling. *Biochem Pharmacol* 2011;82:1391–402.
50 Devallière J, Chatelais M, Fitau J, *et al*. LNK (SH2B3) is a key regulator of integrin signaling in endothelial cells and targets α-parvin to control cell adhesion and migration. *Faseb J* 2012;26:2592–606.
51 Peden DB, Hohman R, Brown ME, *et al*. Uric acid is a major antioxidant in human nasal airway secretions. *Proc Natl Acad Sci U S A* 1990;87:7638–42.

52 Kool M, Willart MA, van Nimwegen M, *et al*. An unexpected role for uric acid as an inducer of T helper 2 cell immunity to inhaled antigens and inflammatory mediator of allergic asthma. *Immunity* 2011;34:527–40.
53 Hara K, Iijima K, Elias MK, *et al*. Airway uric acid is a sensor of inhaled protease allergens and initiates type 2 immune responses in respiratory mucosa. *J Immunol* 2014;192:4032–42.
54 Webb R, Jeffries M, Sawalha AH. Uric acid directly promotes human T-cell activation. *Am J Med Sci* 2009;337:23–7.
55 Gold MJ, Hiebert PR, Park HY, *et al*. Mucosal production of uric acid by airway epithelial cells contributes to particulate matter-induced allergic sensitization. *Mucosal Immunol* 2016;9:809–20.
56 Horsfall LJ, Nazareth I, Petersen I. Serum uric acid and the risk of respiratory disease: a population-based cohort study. *Thorax* 2014;69:1021–6.
57 Aida Y, Shibata Y, Osaka D, *et al*. The relationship between serum uric acid and spirometric values in participants in a health check: the Takahata study. *Int J Med Sci* 2011;8:470–8.
58 Kobylecki CJ, Vedel-Krogh S, Afzal S, *et al*. Plasma urate, lung function and chronic obstructive pulmonary disease: a Mendelian randomisation study in 114 979 individuals from the general population. *Thorax* 2017. 10.1136/thoraxjnl-2017-210273. [Epub ahead of print 29 Nov 2017].
59 Denny JC, Ritchie MD, Basford MA, *et al*. PheWAS: demonstrating the feasibility of a phenome-wide scan to discover gene-disease associations. *Bioinformatics* 2010;26:1205–10.
60 Cortes A, Dendrou CA, Motyer A, *et al*. Bayesian analysis of genetic association across tree-structured routine healthcare data in the UK Biobank. *Nat Genet* 2017;49:1311–8.
61 Canela-Xandri O, Rawlik K, Tenesa A. An atlas of genetic associations in UK Biobank. *bioRxiv* 2017.
62 Latourte A, Soumaré A, Bardin T, *et al*. Uric acid and incident dementia over 12 years of follow-up: a population-based cohort study. *Ann Rheum Dis* 2017:e210767.
63 Khan AA, Quinn TJ, Hewitt J, *et al*. Serum uric acid level and association with cognitive impairment and dementia: systematic review and meta-analysis. *Age* 2016;38:16.
64 Robinson PC, Merriman TR, Herbison P, *et al*. Hospital admissions associated with gout and their comorbidities in New Zealand and England 1999-2009. *Rheumatology* 2013;52:118–26.
65 Brion MJ, Shakhbazov K, Visscher PM. Calculating statistical power in Mendelian randomization studies. *Int J Epidemiol* 2013;42:1497–501.

CONCISE REPORT

Relationship between serum urate concentration and clinically evident incident gout: an individual participant data analysis

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ABSTRACT

Objectives To provide estimates of the cumulative incidence of gout according to baseline serum urate.

Methods Using individual participant data from four publicly available cohorts (Atherosclerosis Risk in Communities Study, Coronary Artery Risk Development in Young Adults Study, and both the Original and Offspring cohorts of the Framingham Heart Study), the cumulative incidence of clinically evident gout was calculated according to baseline serum urate category. Cox proportional hazards modelling was used to evaluate the relation of baseline urate categories to risk of incident gout.

Results This analysis included 18 889 participants who were gout-free at baseline, with mean (SD) 11.2 (4.2) years and 212 363 total patient-years of follow-up. The cumulative incidence at each time point varied according to baseline serum urate concentrations, with 15-year cumulative incidence (95% CI) ranging from 1.1% (0.9 to 1.4) for <6 mg/dL to 49% (31 to 67) for ≥10 mg/dL. Compared with baseline serum urate <6 mg/dL, the adjusted HR for baseline serum urate 6.0–6.9 mg/dL was 2.7, for 7.0–7.9 mg/dL was 6.6, for 8.0–8.9 mg/dL was 15, for 9.0–9.9 mg/dL was 30, and for ≥10 mg/dL was 64.

Conclusions Serum urate level is a strong non-linear concentration-dependent predictor of incident gout. Nonetheless, only about half of those with serum urate concentrations ≥10mg/dL develop clinically evident gout over 15 years, implying a role for prolonged hyperuricaemia and additional factors in the pathogenesis of gout.

Elevated serum urate concentration (hyperuricaemia) is considered to be a key risk factor for developing gout.[1] However, a wide variation in estimates of risk has been reported depending on different published studies.[2–5] The prevalence of hyperuricaemia is increasing.[6] Understanding the consequences and risks of hyperuricaemia is important, both from a health planning perspective and to inform advice given to individuals with hyperuricaemia. The aim of this analysis of individual participant data was to examine the relationship between serum urate concentration and clinically evident incident gout, and specifically to provide estimates of the cumulative incidence of gout according to baseline serum urate concentrations.

METHODS

The eligibility criteria for inclusion in this individual participant data analysis were any longitudinal observational study with the following data: publicly available patient-level data; incident gout data available; gout defined using recognised classification criteria, or doctor diagnosis, or patient self-report of disease, or self-report of doctor diagnosis; serum urate measured prior to assessment for incident gout; and a minimum duration of 3 years of follow-up.

Studies were identified through a systematic search of PubMed and the Database of Genotype and Phenotype (DbGaP) on 11 June 2016, searching the following terms: 'gout and uric'. All articles published since 1 January 1980 were reviewed. The reference lists from comprehensive reviews and identified prospective studies were manually searched. The results of the various searches were reviewed by two authors (ND and TRM). Individual participant-level data were accessed from DbGaP (Project #834: The genetic basis of gout). Full details about data extraction are shown in online supplementary methods.

For all cohorts, the baseline visit was selected as the first examination with both serum urate-specific and gout-specific data publicly available. The cumulative incidence of gout was calculated for the entire group per mg/dL serum urate category (primary analysis), and then for men and women separately (secondary analysis), over 3 years, 5 years, 10 years and 15 years. The incidence results were summarised as both cumulative incidence (%) and as the number of incident cases per 1000 person-years.

The time to onset of gout for each stratum of serum urate at baseline was examined using Kaplan-Meier estimates. Cox proportional hazard multivariable modelling was employed to model the hazard of gout for each category of serum urate at baseline (compared with <6 mg/dL); this analysis included age, sex, ethnicity and cohort as covariates. Where available, the date of gout diagnosis was used in the primary analysis, and if not available examination date at which gout was first reported. In sensitivity analyses, the examination date at which incident gout was recorded and the interaction term of cohort × baseline serum urate were included in the models. No imputation for missing data was undertaken. All analyses were undertaken using the Statistical Package for Social Sciences (SPSS) V.24.0 software.

RESULTS

Search results

Search results are shown in online supplementary figure 1. Four cohorts with publicly available data fulfilled the inclusion criteria and were included in this analysis: Atherosclerosis Risk in Communities Study (ARIC), Coronary Artery Risk Development in Young Adults Study (CARDIA), and both the Original and Offspring cohorts of the Framingham Heart Study (FHS).

Cohort and participant characteristics

Full methods and characteristics of the cohorts have been reported previously.[7–10] Detailed cohort and participant details included in this analysis are shown in online supplementary table 1. For both ARIC and CARDIA, the diagnosis date of incident gout was available. For the two FHS cohorts, only the examination date at which incident gout was recorded was available. There were a total of 18 889 participants who were gout-free at baseline, with mean (SD) 11.2 (4.2) years and 212 363 total patient-years of follow-up. Overall, there were 8280 men (43.8%) in the analysis, and the mean (range) at the time of baseline serum urate testing was 49 (17–85) years.

The ARIC cohort contributed the largest number of participants (n=10 775), followed by CARDIA (n=3470). Similar percentages of men were included in all cohorts (42%–46%). There were differences in age at baseline between the cohorts, with younger participants in CARDIA compared with other cohorts. Ethnicity also differed between cohorts, with predominantly white participants in the FHS cohorts, and both white and African–American participants in the ARIC and CARDIA cohorts. Serum urate concentrations at baseline were higher in the ARIC cohort, compared with the other cohorts.

Cumulative incidence of gout

For all participants, the overall cumulative incidence (95% CI) of gout by 3 years was 0.6% (0.4 to 0.8), by 5 years was 1.1% (0.9 to 1.3), by 10 years was 2.4% (2.2 to 2.6) and by 15 years was 3.2% (2.8 to 3.6) (table 1). The cumulative incidence of gout was lower in women than in men for all time points (table 1).

The cumulative incidence for each time point increased in a non-linear concentration-dependent manner according to baseline serum urate concentration (table 1, figure 1, online supplementary figure 2). By 5 years, the cumulative incidence (95% CI) ranged from 0.33% (0.23 to 0.43) for baseline serum urate <6 mg/dL to 26% (17 to 36) for ≥10 mg/dL (table 1, online supplementary table 2). By 10 years, the cumulative incidence (95% CI) ranged from 0.79% (0.63 to 0.96) for baseline serum urate <6 mg/dL to 40% (29 to 51) for ≥10 mg/dL. The 15-year cumulative incidence (95% CI) ranged from 1.1% (0.90 to 1.4) for baseline serum urate <6 mg/dL to 49% (31 to 67) for ≥10 mg/dL.

Based on the 5-year data, the incidence of gout for those with serum urate ≥7 mg/dL was 9.8/1000 person-years, for ≥8 mg/dL was 20/1000 person-years, for ≥9 mg/dL was 34/1000 person-years and ≥10 mg/dL was 53/1000 person-years (online supplementary tables 3–4).

For women with high serum urate concentrations at baseline, the cumulative incidence of gout was lower by 3 years of follow-up compared with men with the equivalent high serum urate concentrations at baseline (table 1 and online supplementary figure 2). However, for the longer time points (10 years and 15 years), women with high serum urate concentrations at baseline had similar cumulative incidence of gout as men with equivalent high serum urate concentrations.

Risk of developing gout

In the Cox proportional hazard analysis, cohort, male sex, older age, non-white ethnicity and baseline serum urate were independent predictors for incident gout (table 2). Compared with baseline serum urate <6 mg/dL, the adjusted HR (95% CI) for baseline serum urate 6.0–6.9 mg/dL was 2.7 (2.0 to 3.6), for 7.0–7.9 mg/dL was 6.6 (5.0 to 8.8), for 8.0–8.9 mg/dL was 15 (11 to 20), for 8.0–8.9 mg/dL was 30 (21 to 42) and for ≥10 mg/dL was 64 (43 to 96). Sensitivity analyses did not demonstrate any major differences in the adjusted HRs when examination date or an interaction term of cohort × baseline serum urate was included in the models (online supplementary tables 5–6).

DISCUSSION

This analysis of individual participant data demonstrates that serum urate is a strong non-linear concentration-dependent predictor of clinically evident incident gout. We provide cumulative incidence estimates that may guide discussions with individuals with hyperuricaemia about their risk of developing gout over time.

The results of this analysis including individual participant data for >18 000 people can be compared with smaller studies describing the relationship between serum urate concentrations and development of incident gout. A concentration-dependent relationship between serum urate concentrations and development of incident gout has been reported in other studies,[2–5] with a wide range of estimated incidence values. Our individual participant data analysis from four separate cohorts has shown a similar incidence for men as the Normative Aging Study and a much lower annual incidence than reported in other studies.[4 5] Our data indicate that the absolute risk of incident gout in those with modestly elevated serum urate concentrations is low, and even those with very high serum urate concentrations do not invariably develop gout, even over a prolonged period of follow-up. These observations imply a role for additional factors in the pathogenesis of gout; such factors would include inhibitors or promoters of crystal formation in the presence of elevated tissue urate concentrations,[11] and/or genetic and environmental factors that influence the inflammatory response to deposited crystals.

We also show in the Cox proportional hazard analysis that, in addition to serum urate concentrations, sex, age and ethnicity influence the risk of developing gout. While women had lower risk of incident gout compared with men for early time points, the risk according to serum urate level became similar between men and women with longer periods of observation. The duration of exposure to elevated urate concentrations may be different in women, as serum urate concentrations are generally lower before the menopause.[12] Together with the increased risk with older age, these sex findings suggest that the duration of exposure to hyperuricaemia contributes to the development of gout.

There was a small percentage of participants with baseline serum urate below 6 mg/dL who subsequently developed gout. Some of these individuals may have been misdiagnosed with gout, had a falsely low serum urate at the time of testing or had subsequent increases in serum urate. It is also possible that these individuals truly developed gout at a low serum urate concentration, potentially due to local tissue factors that influence urate solubility, crystal nucleation or crystal growth.

Limitations of this study include the variable gout definitions and methods of ascertainment. Importantly, a specific question about gout was recorded at each of the study visits included.

Table 1 Cumulative incidence based on baseline serum urate groups for the entire group, men and women for each follow-up period

Baseline urate (mg/dL)	n	3 years			5 years			10 years			15 years		
		Incidence (%)	Lower 95% CI	Upper 95% CI	Incidence (%)	Lower 95% CI	Upper 95% CI	Incidence (%)	Lower 95% CI	Upper 95% CI	Incidence (%)	Lower 95% CI	Upper 95% CI
All participants													
<6.0	12 103	0.21	0.13	0.29	0.33	0.23	0.43	0.79	0.63	0.96	1.12	0.90	1.35
6.0–6.9	3848	0.37	0.18	0.56	0.66	0.40	0.92	1.98	1.47	2.48	3.70	2.79	4.62
7.0–7.9	1870	0.92	0.49	1.36	1.91	1.28	2.54	6.37	4.94	7.80	9.00	6.93	11.06
8.0–8.9	758	4.00	2.59	5.40	6.94	5.12	8.76	11.32	8.84	13.79	16.28	12.00	20.55
9.0–9.9	230	8.31	4.73	11.89	14.02	9.51	18.52	24.18	16.55	31.82	35.21	21.94	48.48
≥10.0	80	10.00	3.43	16.57	26.25	16.61	35.89	40.00	29.26	50.74	48.57	30.50	66.64
Overall	18 889	0.60	0.40	0.80	1.10	0.90	1.30	2.40	2.20	2.60	3.20	2.81	3.59
Men													
<6.0	3538	0.35	0.15	0.54	0.52	0.28	0.77	1.04	0.69	1.40	1.55	1.05	2.04
6.0–6.9	2563	0.44	0.18	0.69	0.80	0.45	1.14	2.12	1.49	2.76	4.04	2.94	5.14
7.0–7.9	1388	0.95	0.44	1.46	2.06	1.30	2.81	5.51	4.10	6.91	8.23	6.07	10.38
8.0–8.9	581	5.03	3.25	6.82	7.82	5.63	10.02	11.51	8.87	14.15	15.69	11.31	20.07
9.0–9.9	161	10.03	5.37	14.70	13.81	8.45	19.17	25.41	16.35	34.47	34.18	20.24	48.13
≥10.0	49	14.29	4.49	24.08	28.57	15.92	41.22	36.73	23.24	50.23	47.28	25.31	69.24
Overall	8280	1.08	0.86	1.31	1.82	1.53	2.11	3.50	3.07	3.93	4.94	4.34	5.54
Women													
<6.0	8565	0.15	0.07	0.24	0.25	0.14	0.36	0.69	0.51	0.88	0.95	0.70	1.20
6.0–6.9	1285	0.24	0.00	0.51	0.40	0.05	0.75	1.58	0.88	2.29	2.53	1.04	4.02
7.0–7.9	482	0.84	0.02	1.67	1.49	0.39	2.59	10.47	5.50	15.44	12.42	6.26	18.57
8.0–8.9	177	0.57	0.00	1.68	4.05	1.11	6.98	11.08	4.36	17.80	20.44	6.78	34.10
9.0–9.9	69	4.35	0.00	9.16	14.49	6.19	22.80	18.84	9.61	28.07	39.13	4.00	74.26
≥10.0	31	3.23	0.00	9.45	22.58	7.86	37.30	45.16	27.64	62.68	45.16	27.64	62.68
Overall	10 609	0.24	0.15	0.33	0.55	0.41	0.69	1.51	1.25	1.76	1.90	1.58	2.22

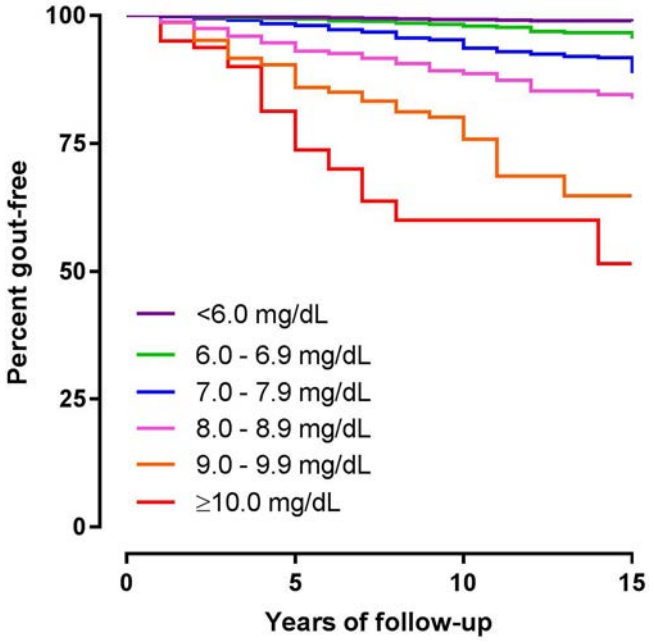

Figure 1 Kaplan-Meier plot showing the percentage of participants who were gout-free over the follow-up period, based on baseline serum urate categories in mg/dL.

In a previous large, multinational study, we have demonstrated that self-report of gout performs very well as a survey definition of gout, when compared with crystal identification as the gold standard.[13] Our sensitivity analysis did not indicate a major difference in results depending on whether the diagnosis date or examination date was used to estimate the date of incident gout. Baseline serum urate concentrations were measured between 1972 and 1989, and it is possible that changes in the environment over the last 30 years may influence contemporary incidence estimates. In addition, the incidence estimates were based on a single urate concentration at the baseline time period, and fluctuation of serum urate over time may also influence the risk of developing disease. This study did not include other endpoints associated with hyperuricaemia, such as hypertension,[14] chronic kidney disease[15] or cardiovascular disease,[16] and consideration of these conditions may also be of relevance when counselling people with elevated serum urate concentrations. The study addressed clinically evident gout as an outcome and was not able to examine the influence of subclinical monosodium urate (MSU) crystal deposition, which is commonly present in people with asymptomatic hyperuricaemia[17 18] and is associated with more severe coronary artery disease[19]; future prospective studies including advanced imaging methods such as ultrasound or dual-energy CT will be instructive to address this issue.

In summary, this analysis provides cumulative incidence estimates to guide discussions with individuals with elevated serum urate concentrations about their risk of developing gout over time. Although serum urate is a strong concentration-dependent risk factor for developing incident gout, most people with hyperuricaemia do not develop clinically evident gout, even with prolonged periods of observation.

Table 2 Cox proportional hazard model analysis including cohort, age, sex, ethnicity and baseline serum urate concentration

	HR	Lower 95% CI	Upper 95% CI	P value
Cohort				
ARIC	Ref	–	–	–
CARDIA	1.69	0.77	3.68	0.19
FHS Original	3.35	2.49	4.49	< 0.001
FHS Offspring	1.81	1.26	2.59	0.001
Sex				
Male	–	–	–	–
Female	0.70	0.58	0.86	0.001
Age				
<30 years	Ref	–	–	–
30–39 years	1.61	0.84	3.10	0.16
40–49 years	3.48	1.58	7.64	0.002
50–59 years	3.64	1.64	8.08	0.002
60–69 years	3.67	1.61	8.33	0.002
70–79 years	4.40	1.77	10.94	0.001
≥80 years	6.10	1.82	20.46	0.003
Ethnicity				
Non-white	Ref	–	–	–
White	0.53	0.42	0.67	<0.001
Baseline serum urate				
<6.0 mg/dL	Ref	–	–	–
6.0–6.9 mg/dL	2.69	2.03	3.57	<0.001
7.0–7.9 mg/dL	6.64	5.04	8.77	<0.001
8.0–8.9 mg/dL	14.92	11.06	20.13	<0.001
9.0–9.9 mg/dL	29.66	20.79	42.31	<0.001
≥10.0 mg/dL	63.96	42.54	96.16	<0.001

ARIC, Atherosclerosis Risk in Communities Study; CARDIA, Coronary Artery Risk Development in Young Adults Study; FHS, Framingham Heart Study; Ref, reference.

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Contributors ND conceived and planned the study, interpreted the data, and wrote the first draft of the manuscript. ND is responsible for the overall content of the manuscript as guarantor. AP-G contributed to analysis planning, data management and manuscript drafting. CF analysed the data and contributed to analysis planning and manuscript drafting. TN, WJT and TRM contributed to study conception and planning, data interpretation, and manuscript drafting.

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REFERENCES

1 Garrod AB. Observations on certain pathological conditions of the blood and urine in gout. *Trans M-Chir Soc Edinburgh* 1848;31:83–97.
2 Duskin-Bitan H, Cohen E, Goldberg E, *et al*. The degree of asymptomatic hyperuricemia and the risk of gout. A retrospective analysis of a large cohort. *Clin Rheumatol* 2014;33:549–53.
3 Campion EW, Glynn RJ, DeLabry LO. Asymptomatic hyperuricemia. Risks and consequences in the Normative Aging Study. *Am J Med* 1987;82:421–6.
4 Bhole V, de Vera M, Rahman MM, *et al*. Epidemiology of gout in women: Fifty-two-year followup of a prospective cohort. *Arthritis Rheum* 2010;62:1069–76.
5 Lin KC, Lin HY, Chou P. The interaction between uric acid level and other risk factors on the development of gout among asymptomatic hyperuricemic men in a prospective study. *J Rheumatol* 2000;27:1501–5.
6 Zhu Y, Pandya BJ, Choi HK. Prevalence of gout and hyperuricemia in the US general population: the National Health and Nutrition Examination Survey 2007-2008. *Arthritis Rheum* 2011;63:3136–41.
7 The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. The ARIC investigators. *Am J Epidemiol* 1989;129:687–702.
8 Friedman GD, Cutter GR, Donahue RP, *et al*. CARDIA: study design, recruitment, and some characteristics of the examined subjects. *J Clin Epidemiol* 1988;41:1105–16.
9 Dawber TR, Meadors GF, MOORE FE. Epidemiological approaches to heart disease: the Framingham Study. *Am J Public Health Nations Health* 1951;41:279–86.
10 Feinleib M, Kannel WB, Garrison RJ, *et al*. The Framingham Offspring Study. Design and preliminary data. *Prev Med* 1975;4:518–25.
11 McGill NW, Dieppe PA. Evidence for a promoter of urate crystal formation in gouty synovial fluid. *Ann Rheum Dis* 1991;50:558–61.
12 Hak AE, Choi HK, Menopause CHK. Menopause, postmenopausal hormone use and serum uric acid levels in US women--the Third National Health and Nutrition Examination Survey. *Arthritis Res Ther* 2008;10:R116.
13 Dalbeth N, Schumacher HR, Fransen J, *et al*. Survey Definitions of Gout for Epidemiologic Studies: Comparison With Crystal Identification as the Gold Standard. *Arthritis Care Res* 2016;68:1894–8.
14 Wang J, Qin T, Chen J, *et al*. Hyperuricemia and risk of incident hypertension: a systematic review and meta-analysis of observational studies. *PLoS One* 2014;9:e114259.
15 Li L, Yang C, Zhao Y, *et al*. Is hyperuricemia an independent risk factor for new-onset chronic kidney disease?: A systematic review and meta-analysis based on observational cohort studies. *BMC Nephrol* 2014;15:122.
16 Li M, Hu X, Fan Y, *et al*. Hyperuricemia and the risk for coronary heart disease morbidity and mortality a systematic review and dose-response meta-analysis. *Sci Rep* 2016;6:19520.
17 Howard RG, Pillinger MH, Gyftopoulos S, *et al*. Reproducibility of musculoskeletal ultrasound for determining monosodium urate deposition: concordance between readers. *Arthritis Care Res* 2011;63:1456–62.
18 Dalbeth N, House ME, Aati O, *et al*. Urate crystal deposition in asymptomatic hyperuricaemia and symptomatic gout: a dual energy CT study. *Ann Rheum Dis* 2015;74:908–11.
19 Andrés M, Quintanilla MA, Sivera F, *et al*. Silent monosodium urate crystal deposits are associated with severe coronary calcification in asymptomatic hyperuricemia: an exploratory study. *Arthritis Rheumatol* 2016;68:1531–9.

EXTENDED REPORT

Hypermetabolic macrophages in rheumatoid arthritis and coronary artery disease due to glycogen synthase kinase 3b inactivation

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ABSTRACT

Objectives Accelerated atherosclerotic disease typically complicates rheumatoid arthritis (RA), leading to premature cardiovascular death. Inflammatory macrophages are key effector cells in both rheumatoid synovitis and the plaques of coronary artery disease (CAD). Whether both diseases share macrophage-dependent pathogenic mechanisms is unknown.

Methods Patients with RA or CAD (at least one myocardial infarction) and healthy age-matched controls were recruited into the study. Peripheral blood CD14+ monocytes were differentiated into macrophages. Metabolic profiles were assessed by Seahorse Analyzer, intracellular ATP concentrations were quantified and mitochondrial protein localisation was determined by confocal image analysis.

Results In macrophages from patients with RA or CAD, mitochondria consumed more oxygen, generated more ATP and built tight interorganelle connections with the endoplasmic reticulum, forming mitochondria-associated membranes (MAM). Calcium transfer through MAM sites sustained mitochondrial hyperactivity and was dependent on inactivation of glycogen synthase kinase 3b (GSK3b), a serine/threonine kinase functioning as a metabolic switch. In patient-derived macrophages, inactivated pGSK3b-Ser9 co-precipitated with the mitochondrial fraction. Immunostaining of atherosclerotic plaques and synovial lesions confirmed that most macrophages had inactivated GSK3b. MAM formation and GSK3b inactivation sustained production of the collagenase cathepsin K, a macrophage effector function closely correlated with clinical disease activity in RA and CAD.

Conclusions Re-organisation of the macrophage metabolism in patients with RA and CAD drives unopposed oxygen consumption and ultimately, excessive production of tissue-destructive enzymes. The underlying molecular defect relates to the deactivation of GSK3b, which controls mitochondrial fuel influx and as such represents a potential therapeutic target for anti-inflammatory therapy.

INTRODUCTION

Unstable atherosclerotic plaques display numerous similarities with bone-erosive synovial lesions in the rheumatoid joint, especially the dominant role of inflammatory macrophages.[1–3] By amplifying inflammatory circuits and directly contributing to atherosclerotic plaque destabilisation,[4] macrophages may contribute to the enhanced cardiovascular risk in patients with rheumatoid arthritis (RA). Despite improved disease control, patients with RA have a twofold increased risk for myocardial infarction[5] with traditional cardiovascular risk factors explaining this only partially.[6] Even with mild disease activity, RA increases cardiovascular mortality substantially and is an independent risk factor for cardiovascular complications.[7 8] Premature cardiovascular death is the major cause of mortality in patients with RA.[9] We hypothesised that macrophages in patients with RA and CAD share functional abnormalities that promote inflammatory disease.

Macrophages in arthritic joints are hypermetabolic and produce excess amounts of succinate, which is sensed via GPR91 to amplify cytokine release.[10] Also, RA macrophages express high amounts of the glycolytic enzyme α-enolase, which is recognised by autoantibodies to induce cytokine production.[11] Macrophages of patients with coronary artery disease (CAD) excel in glucose uptake and overexpress the glycolytic enzyme pyruvate kinase M2, which functions as a protein kinase, phosphorylates signal transducer and activator of transcription 3 (STAT3) and boosts cytokine production.[12] The glycolytic intermediate pyruvate controls cell surface expression of immunoinhibitory programmed death-ligand 1 (PD-L1) in CAD macrophages, thus weakening protective immunity.[13]

The kinase glycogen synthase kinase 3b (GSK3b), first named for the enzyme's contribution to glycogen storage,[14] is now recognised for affecting multiple signalling pathways.[15] In most cell types, including macrophages, GSK3b is constitutively active[16] and serine-9 phosphorylation results in its inactivation.[17] GSK3b's function is cell-type and context-dependent,[16 18] but the kinase was recently implicated in metabolic regulation.[19–22]

Mitochondria-associated membranes (MAM) are physical contacts between the endoplasmic reticulum (ER) and mitochondria. ER-to-mitochondria calcium transfer stimulates calcium-sensitive tricarboxylic acid (TCA) cycle enzymes to promote mitochondrial metabolism[23] and calcium can directly activate the mitochondrial ATPase.[24]

Here, we report that macrophages from patients who have RA or CAD share a molecular phenotype of mitochondrial hyperactivation, which is

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mechanistically linked to GSK3b de-activation. Inactivated GSK3b co-precipitates with mitochondria from patient-derived but not from healthy macrophages. Functional consequences include MAM formation, enhancing mitochondrial activation. MAM function and mitochondrial activation were linked to macrophage effector functions, specifically the release of the potent collagenase cathepsin K. The link between the hypermetabolic state and the tissue-damaging potential of inflammatory macrophages identifies GSK3b as a potential target to correct inappropriate immunity in RA and to prevent progression of atherosclerosis.

METHODS

Patients and controls

The study population included 74 patients with RA, 68 patients with CAD and 50 age-matched healthy controls. Patients with RA fulfilled the 2010 diagnostic criteria and were positive for anti-cyclic citrullinated peptide (CCP) antibodies or for rheumatoid factor and were enrolled between January 2016 and December 2017. In parallel, patients with CAD who had at least one documented myocardial infarction (>90 days after the ischaemic event) were recruited. Clinical characteristics are given in online supplementary tables S1 and S2. Demographically matched healthy individuals were obtained from the Stanford Blood Center. They had no history of autoimmune disease, cancer, chronic viral infection or any other inflammatory syndrome. Written informed consent was obtained as appropriate.

Cells and culture

Peripheral blood mononuclear cells (PBMCs) were isolated using Lymphoprep (STEMCELL Technologies). CD14+ cells were differentiated into macrophages in Roswell Park Memorial Institute (RPMI) 1640 medium (Life Technologies) supplemented with 20 ng/mL macrophage colony-stimulating factor (M-CSF) (eBioscience) and 10% fetal bovine serum (FBS) (Lonza) for 5 days as reported previously.[12] Macrophages were differentiated by stimulation with 100 U/mL IFN-γ (Sino Biologicals) and 100 ng/mL lipopolysaccharide (LPS) (Sigma-Aldrich). Macrophages were detached using StemPro Accutase Cell Dissociation (Life Technologies, Thermo Fisher). For inhibition of GSK3b, macrophages were treated with SB216763 (10 µM; Abcam). To inhibit mitochondrial calcium uptake, cells were treated with Ru360 (10 µM; Sigma-Aldrich).

Statistical analysis

Unpaired t-test was applied when comparing groups, and paired t-test when analysing paired data. Pearson correlation coefficient was used for correlation analysis. All data analysed by Prism V.6 (GraphPad).

Detailed methods are provided in the online supplementary materials.

RESULTS

Increased mitochondrial activity and ATP production in macrophages from patients with RA and CAD

Macrophages were generated from two patient cohorts: patients with autoantibody-positive RA and patients who had CAD-induced cardiac ischaemia. To test the metabolic competence, we analysed mitochondrial oxygen consumption rates (OCR) and extracellular acidification rates (ECAR) by Seahorse extracellular flux assays (figure 1A). Mitochondria from patient-derived macrophages consumed significantly more oxygen than those from healthy age-matched controls (basal OCR, figure 1B). ATP-coupled OCR, probed by oligomycin inhibition of mitochondrial ATP synthase, was higher in disease macrophages, with CAD macrophages outpacing RA macrophages (figure 1C). Similarly, maximal respiration, tested by uncoupling the electron transfer chain with carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP), was higher in RA and CAD macrophages than in controls (figure 1D). (figure 1E=). Mitochondria from RA and CAD macrophages had explicitly more reserve capacity to work against imminent energy deficits (figure 1E).(figure 1D). In parallel, patient-derived cells intensified glycolysis, captured as higher ECAR (figure 1F). All cohorts had similar ratios of glycolysis to mitochondrial respiration (ECAR/OCR, figure 1G). In line with higher oxygen consumption, mitochondrial membrane potentials, responsible for driving electron transport (figure 1H,I) and generation of reactive oxygen species (ROS) (figure 1J,K) were higher in RA and CAD macrophages. Mitochondrial hyperactivity was already obvious in freshly isolated monocytes (online supplementary figure S1A,B). Increased mitochondrial respiration in the patients' cells resulted in higher ATP production (figure 1L). Together, these data demonstrated that patient-derived macrophages are in a state of heightened mitochondrial activity.

Enhanced MAM formation and intensified ER mitochondria calcium transfer in RA and CAD macrophages

MAM are specialised organelle structures connecting mitochondria and the ER to transport lipids and calcium.[25 26] To gain information about the structural intactness of mitochondria, we performed co-localisation studies of proteins involved in tethering mitochondria to the ER. Inositol 1,4,5-trisphosphate receptors (IP3R), calcium channels in the ER outer membrane, connect to mitochondrial membranes through 75 KDa glucose-regulated protein (GRP75). Confocal microscopy revealed IP3R/GRP75 co-localisation to a higher degree in RA and CAD macrophages compared with healthy samples (figure 2A). Quantification confirmed a significantly higher protein co-localisation, and thus MAM formation, in patient-derived macrophages (figure 2B). The overall signal for IP3R and GRP75 was indistinguishable in the three study cohorts (online supplementary figure S2), suggesting that increased co-localisation of the markers was reflective of structural rearrangements.

To provide biochemical evidence for accelerated MAM formation, we analysed mitochondrial calcium uptake. Representative curves of mitochondrial calcium influx (figure 2C) illustrated greater calcium uptake in patient-derived macrophages. Quantification (relative increase peak value−baseline value) verified higher calcium uptake through the MAM contact sites in patient-derived cells compared with control cells (figure 2D). Spontaneously increased calcium flux was confirmed for freshly isolated monocytes (online supplementary figure S1C). To assess the impact of calcium import on mitochondrial activity, RA and CAD macrophages were treated with Ru360, a specific inhibitor of mitochondrial calcium uptake. Disrupting calcium flux caused a decline in mitochondrial membrane potential (figure 2E), diminished ROS production (figure 2F) and lowered ATP generation (figure 2G).

Thus, the signature of mitochondrial hyperactivity in RA and CAD macrophages is associated with structural adaptations, physically connecting mitochondria and the ER by MAM formation to promote calcium transfer.

GSK3b is deactivated in patient-derived macrophages

Mitochondrial hyperactivity in RA and CAD macrophages raised the question how mitochondria are instructed to upregulate

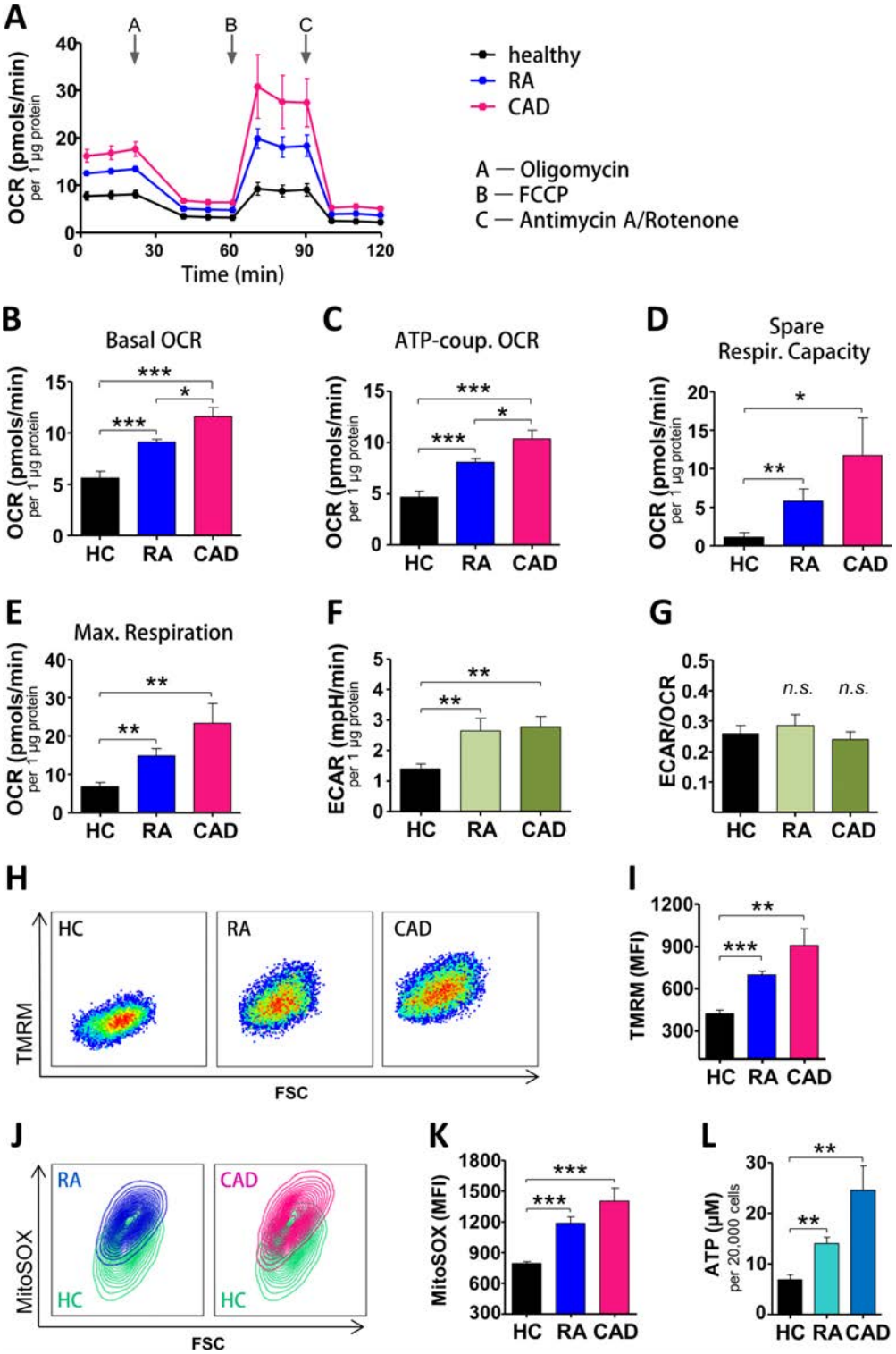

Figure 1 Increased mitochondrial activity and ATP production in macrophages from patients with RA and CAD. (A) Summarised curves of OCR tracings from Seahorse experiments for all study cohorts (HC; n=7. Patients with RA or CAD; n=10 each). Baseline Respiration (B), respiration coupled to ATP production (C), respiratory spare capacity (D) and maximal respiration (E) were calculated based on OCR. (F) Seahorse-derived ECAR values and (G) ECAR-to-OCR ratios. (H,I) Representative dot plots and MFIs from TMRM staining indicative for mitochondrial membrane potential from six samples in each group. (J,K) MitoSOX Red staining indicative for mitochondrial reactive oxygen species. Representative contour plots from RA and CAD macrophages compared with a control sample (green) and summary results from six samples in each group. (L) Intracellular ATP concentrations per 20 000 activated macrophages from six samples in each group. Unpaired t-test was applied. *P<0.05; **P<0.01; ***P<0.001. All bar graphs show mean±SEM. CAD, coronary artery disease; ECAR, extracellular acidification rate; FSC, forward scatter channel; HC, healthy control; MFI, mean fluorescence intensity; OCR, oxygen consumption rate; RA, rheumatoid arthritis; TMRM, tetramethylrhodamine methyl ester.

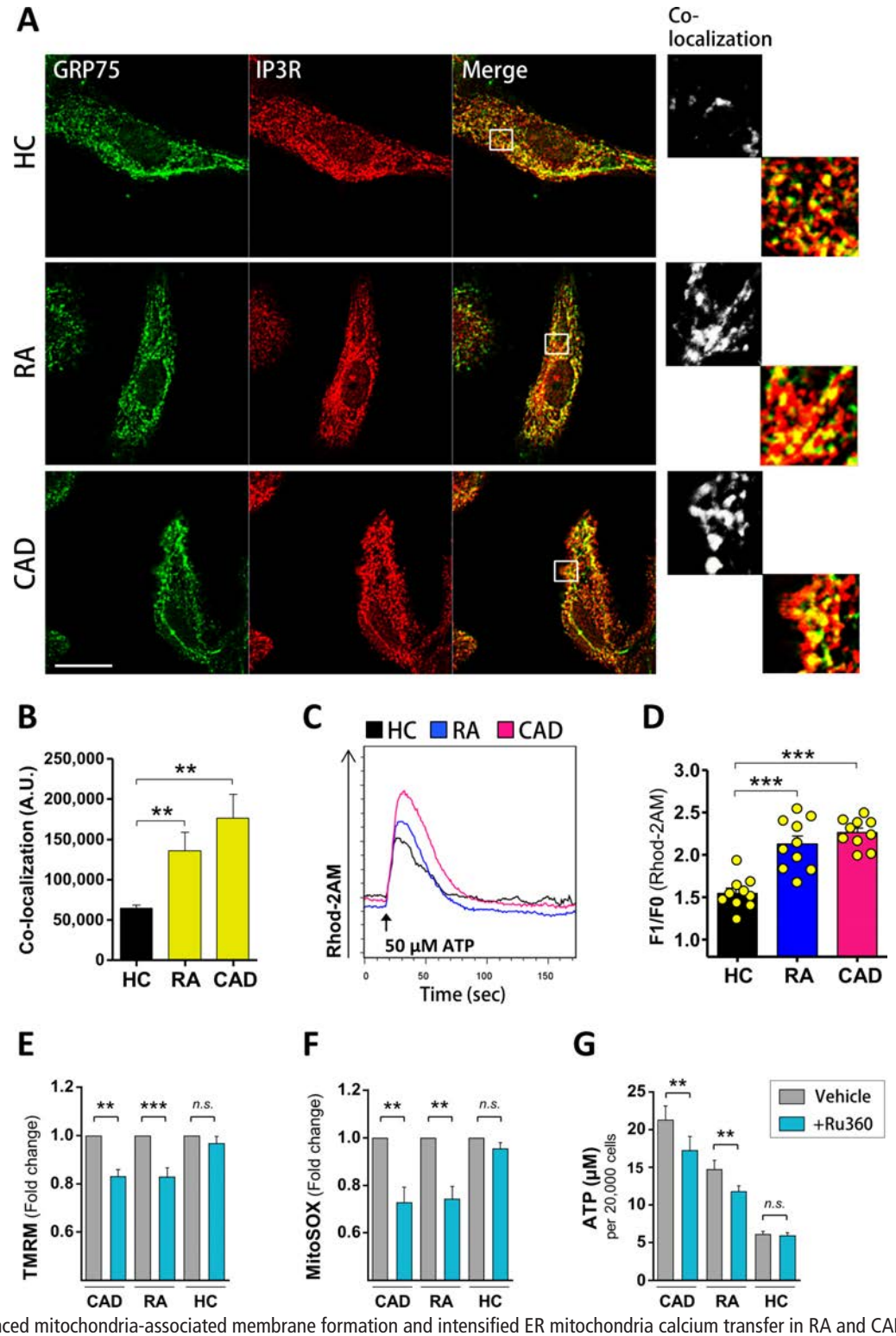

Figure 2 Enhanced mitochondria-associated membrane formation and intensified ER mitochondria calcium transfer in RA and CAD macrophages. (A) Confocal microscopy of activated macrophages stained with anti-GRP75 (green) and anti-IP3R (red). White boxes represent enlarged areas in the right panel. (B) Quantification of GRP75/IP3R co-localisation. Summarised data from six healthy, five RA and five CAD samples. (C) Representative histogram of mitochondrial calcium uptake after calcium was released from the ER with 50 µM ATP; (D) bar graphs summarise results from 10 experiments (peak value F1 divided by baseline level F0). (E–G) Mitochondrial membrane potential (n=7 each group), mitochondrial reactive oxygen species (n=7 each group) and intracellular ATP (n=6 each group) measured after inhibiting mitochondrial calcium influx with Ru360 (10 µM). Unpaired t-test (B–D) and paired t-test (E–H). Scale bar 10 µm. *P<0.05; **P<0.01; ***P<0.001. All bar graphs show mean±SEM. CAD, coronary artery disease; ER, endoplasmic reticulum; GRP75, 75 KDa glucose-regulated protein; HC, healthy control; IP3R, inositol 1,4,5-trisphosphate receptor; RA, rheumatoid arthritis; TMRM, tetramethylrhodamine methyl ester.

multiple of their functional domains. The kinase GSK3b has recently been recognised as a regulator of cellular metabolism.[20–22] In most cell types, including macrophages, GSK3b's kinase function is constitutively active.[16] To test for the activation status, we quantified pGSK3b-Ser9, the primary target site for inactivation. pGSK3b-Ser9 concentrations were already elevated in resting macrophages from patients with RA and CAD (figure 3B), indicating that mitochondria in these cells were primed for higher functional activity. Polarisation with LPS and IFN-γ had a minor impact on the phosphorylation status (figure 3A,B). Confocal imaging of ex vivo macrophages confirmed higher amounts of pGSK3b-Ser9 in patient cells (figure 3C). GSK3b inactivation was already present in freshly isolated monocytes (figure 3D).

To understand whether inactivated GSK3b was physically associated with mitochondrial membranes, we isolated mitochondrial protein fractions and probed for the presence of pGSK3b-Ser9. Deactivated GSK3b accumulated in the mitochondrial fraction of CAD macrophages and was also present in mitochondria of RA macrophages, whereas it was barely detectable in mitochondria from healthy donors (figure 3E). To test whether inhibition of GSK3b impacted MAM formation, we treated macrophages from healthy donors with an inhibitor (SB216763) and detected enhanced MAM-driven calcium transfer, suggestive for a role of GSK3b in regulating mitochondrial-ER communication (figure 3F,G).

Accumulation of β-catenin, a consequence of GSK3b inactivation, was clearly detectable in RA and CAD macrophages (figure 3H). The major upstream kinase to phosphorylate GSK3b is protein kinase B (Akt). We measured serine-473 phosphorylation of Akt and found significantly more activated Akt in patient macrophages (figure 3I).

These data established inactivation of GSK3b as a key event in priming mitochondria for high performance by increased MAM signalling.

GSK3b is deactivated in macrophages infiltrating into RA synovial tissue and into the atherosclerotic plaque

To investigate whether GSK3b inactivation is specific for macrophages in disease lesions, we immunostained inactive pGSK3b-Ser9 in tissue biopsies from RA synovium (figure 4A) and from atherosclerotic atheroma (figure 4B). CD68+ tissue macrophages accumulated in the lining layer of synovitic lesions and contained inactivated GSK3b. Similarly, CD68+ cells in the atherosclerotic plaque stained positive for pGSK3b-Ser9. In contrast, in control sections of liver, colon and lung (figure 4C–E) tissue-infiltrating macrophages stained negative for pGSK3b-Ser9. Liver cholangiocytes stained positive for pGSK3b-Ser9 and serve as positive control. Isotype control staining is shown in online supplementary figure S3. These findings indicate that macrophages positive for pGSK3b-Ser9 localise to disease lesions and support the concept that molecular signatures are shared among ex vivo-generated monocyte-derived macrophages and lesion-infiltrating cells.

GSK3b regulates mitochondrial activity

Structural and functional analysis suggested a mechanistic link between GSK3b deactivation, MAM-driven calcium transfer and mitochondrial hyperactivity (figures 2E,F and 3E,F). We hypothesised that GSK3b inhibition might recapitulate the total metabolic phenotype observed in RA and CAD macrophages.

Macrophages from healthy donors were treated with SB216763 to inhibit GSK3b, which resulted in accumulation of β-catenin (online supplementary figure S4A), confirming functional deactivation of the kinase. Next, we analysed mitochondrial respiration before and after GSK3b inhibition. Seahorse-generated OCR tracings are shown in figure 5A. GSK3b inhibition stimulated mitochondrial respiration, recapitulating the phenotype observed in disease macrophages (figure 5B) and increased maximal respiration(figure 5D). Also, ATP-coupled OCR and spare respiratory capacity increased (figure 5D,E) (figure 5C, E). Finally, treatment with the inhibitor led to gene induction of the main glucose uptake receptor in macrophages, glucose transporter (GLUT)-1 (online supplementary figure S4B) and glycolytic flux increased (figure 5F).

In line with a phenotype of activated mitochondrial respiration, GSK3b inhibition in control macrophages increased mitochondrial membrane potential (figure 5G,H), led to formation of larger amounts of mitochondrial ROS (figure 5I,J) and allowed greater ATP production (figure 5K). These data identify GSK3b inactivation as a key event in reprogramming macrophage metabolism.

Mitochondrial activity and MAM function drive macrophage cathepsin K production

Macrophages are the main facilitator of tissue damage in both RA joints and atherosclerotic plaques.[1 2] We speculated that GSK3b-dependent metabolic reprogramming had functional implications for macrophage effector functions. We analysed transcriptome profiles for collagenases of the cathepsin family previously reported to be involved in vascular pathology.[27] A member of this family is cathepsin K, one of the most potent collagenases described in mammalian cells.[28]

Cathepsin S transcripts did not separate the study cohorts, and cathepsin L was only upregulated in RA macrophages. In contrast, cathepsin K transcripts were strongly upregulated in patient-derived macrophages, being ninefold higher in cells from patients with CAD (figure 6A). To test whether gene induction of cathepsin K resulted in increased collagenase activity, we measured cathepsin K enzyme activity with a specific assay system. In line with transcriptome data, macrophages from patients with RA and CAD showed higher enzymatic activity (figure 6B).

To explore whether cathepsin K activity was related to disease activity in patients, we correlated clinical disease activity scores with in vitro enzyme activity in macrophages. Inflammatory burden in patients with RA was measured with Clinical Disease Activity Index. In patients with CAD, we used the number of stenotic coronary vessels assessed by coronary angiography. In both patient cohorts, more active disease in vivo was correlated with higher cathepsin K activity (figure 6C,D).

To establish a mechanistic connection between cathepsin K and GSK3b activity, we treated cells from healthy donors with SB216763. Transcript levels for the collagenase rose threefold (figure 6E) and cathepsin K activity increased twofold (figure 6B).

Cathepsin K activity is higher in acidic environments.[29] Macrophages possess specialised membrane-bound proton pumps to release H^+ to acidify the extracellular environment. These vacuolar-type H^+-ATPases (V-ATPase) consume mitochondria-generated ATP; they are more effective in energy-rich cells. We explored whether the abundance of ATP in patient macrophages was associated with expression of V-ATPases. Patient-derived cells spontaneously expressed higher transcripts of V-ATPases subunits V0 and V1 (online supplementary figure S5) and GSK3b inactivation in healthy macrophages was followed by marked induction of V-ATPases transcripts (figure 6F, G).

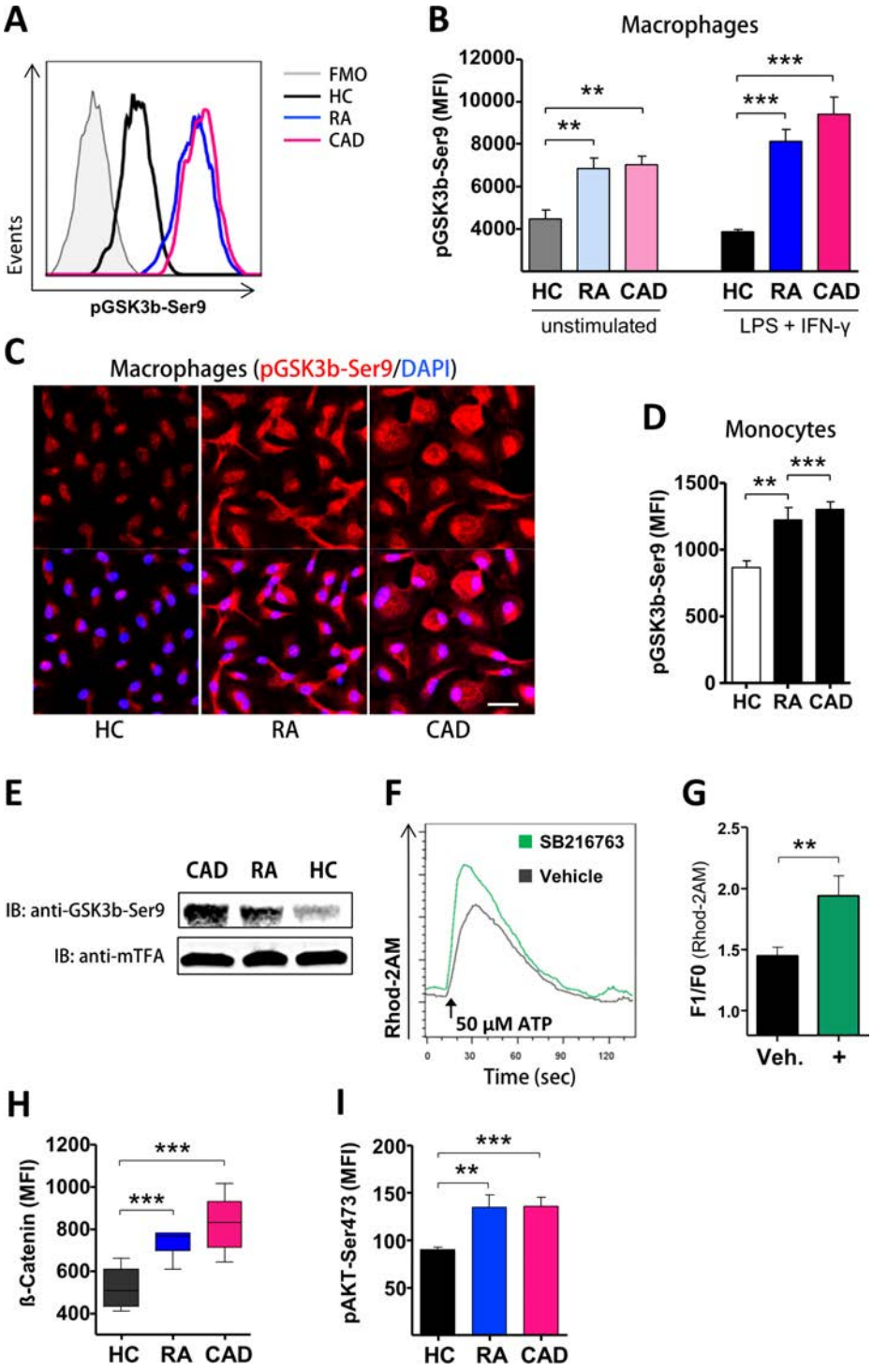

Figure 3 GSK3b is deactivated in patient-derived macrophages. (A) Representative histograms of phosphoflow for pGSK3b-Ser9 from activated macrophages. Bar graph (B) summarises results from six experiments with unstimulated and from eight experiments with activated macrophages. (C) Confocal microscopy of pGSK3b-Ser9 (red) in activated macrophages. Nuclei were localised by DAPI (blue). (D) Quantification of phosphoflow for pGSK3b-Ser9 in fresh monocytes from five experiments. (E) Isolation of mitochondrial fractions and immunoblotting of proteins with antibodies against pGSK3b-Ser9 and mTFA as control protein. Assay was performed three times with four healthy, four RA and four CAD samples. (F) Representative curves and (G) quantification of mitochondrial calcium uptake from six experiments after treatment of macrophages from healthy individuals with inhibitor SB216763 (10 µM). (H) Intracellular accumulation of β-catenin in activated macrophages (n=6 in each group). (I) Phosphoflow of pAkt-S473. Bar graph summarises MFIs from six samples in each group. Unpaired t-test was applied. *P<0.05, **P<0.01, ***P<0.001. All bar graphs show mean±SEM. Scale bar 20 µm. CAD, coronary artery disease; DAPI, 4',6-diamidino-2-phenylindole; FMO, fluorescence minus one control; GSK3b, glycogen synthase kinase 3b; HC, healthy control; mTFA, mitochondrial transcription factor A; MFI, mean fluorescence intensity; RA, rheumatoid arthritis.

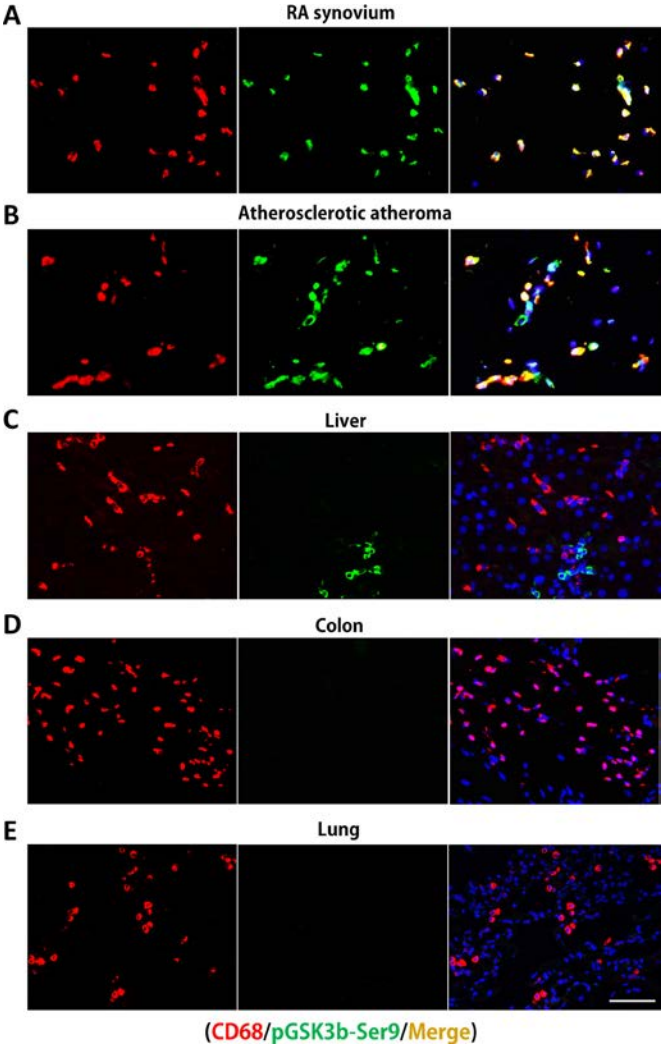

Figure 4 GSK3b is deactivated in macrophages infiltrating into RA synovial tissue and into the atherosclerotic plaque. Tissue sections were immunostained with anti-CD68 (red), anti-pGSK3b-Ser9 (green) and 4',6-diamidino-2-phenylindole (DAPI) (blue) and analysed by fluorescence microscopy. (A) RA synovitis from a 36-year-old female patient with nodular disease taken from the left wrist. (B) Atherosclerotic fibro-calcified atheroma from a 76-year-old male patient with symptomatic carotid stenosis. Control stainings show tissue sections of (C) liver, (D) colon and (E) lung. In liver tissue, cholangiocytes stained positive for pGSK3b-Ser9. Scale bar 50 µm. DAPI, 4',6-diamidino-2-phenylindole; GSK3b, glycogen synthase kinase 3b; RA, rheumatoid arthritis.

We speculated that mitochondrial activity and MAM function support the tissue destructive phenotype of macrophages. To test this hypothesis, we blocked calcium transfer from the ER to mitochondria and measured cathepsin K activity. Abrogation of calcium influx into mitochondria significantly lowered cathepsin K activity in both RA and CAD macrophages (figure 6H, I).

In essence, metabolically active macrophages are able to activate the potent collagenase cathepsin K, and this functional domain depends on MAM function and correlates closely with the disease burden in vivo. Disengagement of GSK3b is a critical step in providing mitochondrial energy generation and a prerequisite for collagenase production and activity.

DISCUSSION

Accelerated atherosclerosis has been observed in several rheumatological diseases. The greatest impact of cardiovascular complications on patient survival can be observed in RA, where premature cardiovascular death is the major cause of mortality.[9] However, molecular mechanisms explaining acceleration of cardiovascular disease are not available. Additionally, it is unclear whether pathways for the progression of atherosclerosis are distinct or shared between patients with and without rheumatological disease. In this study, we demonstrate that macrophages from patients with RA and CAD share a molecular pathway regulated by GSK3b to promote tissue-destructive effector function.

The unexpected finding of this study was the critical role of MAMs in inflammatory macrophages. MAMs are involved in cell death signalling when uncontrolled flooding of the mitochondria with calcium induces apoptosis. Under more physiological conditions, calcium transfer is dosed and tightly regulated to fine-tune mitochondrial activity by activating mitochondrial ATPase[24] and stimulating TCA cycle enzymes.[23] Current data indicate that macrophages from patients with RA and CAD have undergone structural adaptations by increasing physiological MAM contact sites to fulfil their energy demands. Blocking calcium transfer at the MAMs abrogated mitochondrial activation and coordinately inhibited macrophage effector function by mitigating collagenase activity of cathepsin K. Other inflammatory cascades that were previously reported to be dependent on MAM formation include NLRP3 inflammasome activation[30] and formation of RIG-I/MAVS pathogen recognition receptor complexes.[31] Our data place MAMs in the centre of cellular functionality, spanning from host defence to regulation of innate immunity and tissue destruction. In a simplistic model, MAMs serve as connector between cellular energy production and effector function. This mechanism becomes pathogenic when providing energy for tissue-destructive behaviour, thus sustaining conditions of sterile chronic inflammation. Remodelling of the MAM, and with it enhancement of mitochondrial activity, occurs early in the life cycle of macrophages and is already present in precursor monocytes.

The main molecular defect of chronic inflammatory macrophages identified in this study is inactivation of GSK3b, a defect shared by circulating monocytes and differentiated macrophages. GSK3b is constitutively active in most cell types, also in macrophages, and is inhibited in response to stimulation.[32] It is considered to be a regulator of survival and cellular intactness, but its eventual role is strongly cell-type and context-dependent.[16 18] It was reported that active GSK3b is associated with mitochondrial quiescence in drosophila oocytes[21] and that GSK3-deficient B cells showed higher metabolic activity and increased proliferative capacity.[20–22] The current study identifies inactivation of GSK3b as master regulator of metabolic reprogramming in macrophages. Phosphorylation of downstream targets by active GSK3b usually provides an inhibitory effect; this regulatory control of GSK3b in preventing mitochondrial hyperactivity is lost in RA and CAD macrophages. GSK3b-Ser9 localises to mitochondria and stabilises calcium transfer at MAM contact sites, a mechanism necessary to increase ATP production and to drive tissue-destructive effector function. Importantly, this pathway is shared between macrophages from patients with RA and CAD, indicating a more universal validity in chronic inflammatory diseases. Hypermetabolic macrophages could be induced by SB216763 treatment of healthy cells, a small molecule inhibitor for GSK3b and GSK3a. The functional status of GSK3a was not examined, but the consistent increase in inactivated GSK3b by flow cytometry, immunostaining and immunoblotting in patient samples focused attention to GSK3b.

Inactivation of GSK3b had direct functional consequences: it released transcriptional suppression of cathepsin K, a highly

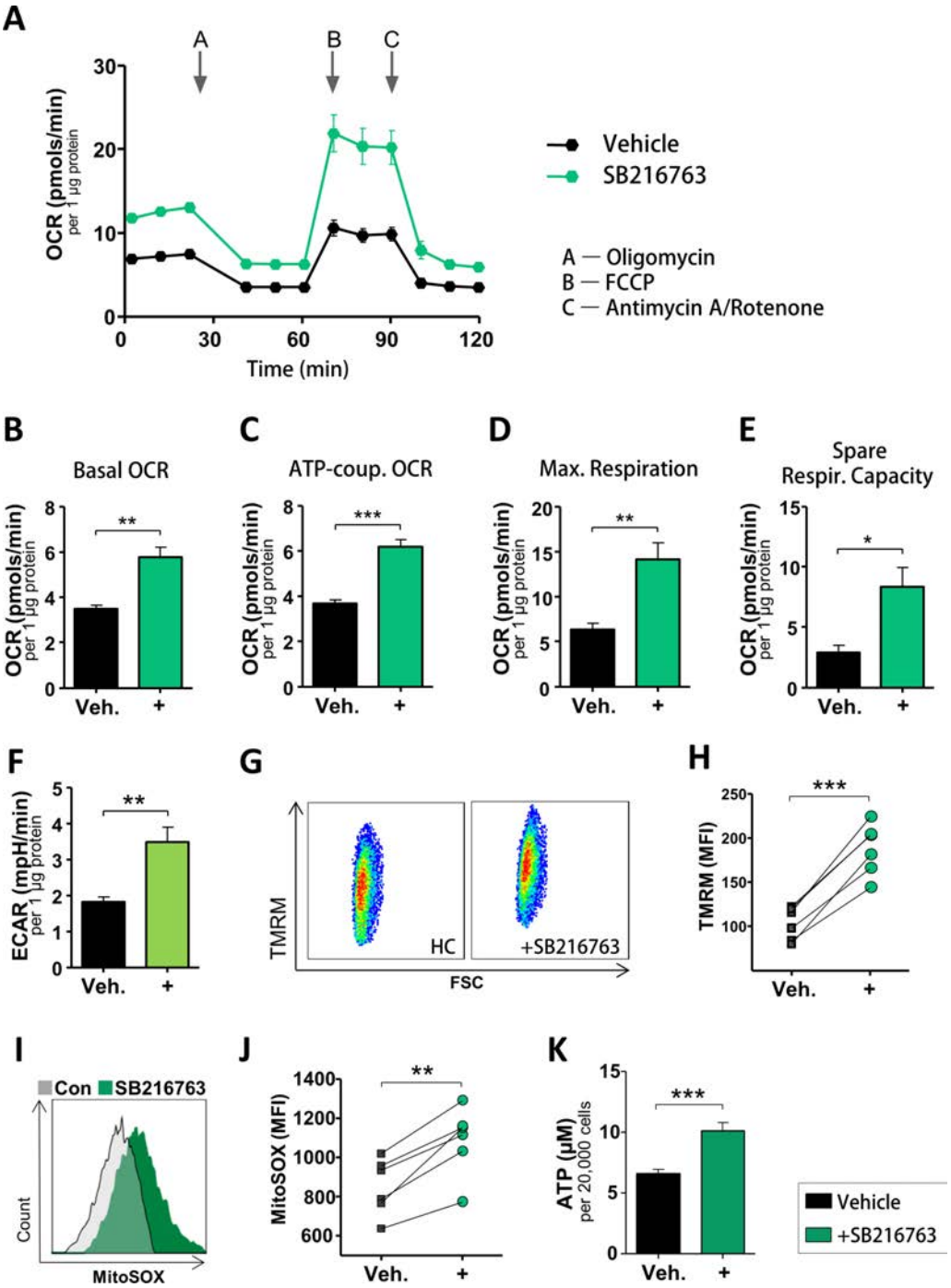

Figure 5 Glycogen synthase kinase 3b regulates mitochondrial activity. OCRs (A–E) and ECARs (F) of macrophages from healthy donors (n=7) were measured as in figure 1 after they were pretreated with vehicle or the inhibitor SB216763 (10 µM, 24 hours). (G) Representative dot plot and (H) summarising bar graph of the quantification of mitochondrial membrane potential (n=6). (I) Representative histogram of mitochondrial reactive oxygen species and (J) summarised results from six experiments. (K) Intracellular ATP concentrations in 20 000 activated macrophages (n=8). All data are mean±SEM. Paired t-test was applied. *P<0.05; **P<0.01; ***P<0.001. ECAR, extracellular acidification rate; FSC, forward scatter channel; HC, healthy control; MFI, mean fluorescence intensity; OCR, oxygen consumption rate; TMRM, tetramethylrhodamine methyl ester.

potent collagenase[28] that is highly expressed in atherosclerotic lesions and in abdominal aortic aneurysms.[33 34] In murine models, cathepsin K deficiency led to smaller atherosclerotic plaques with increased lesion stability.[35] In macrophages examined here, upregulation of cathepsin K was coupled to metabolic hyperactivity and induction of other membrane channels, all functioning in concert to achieve optimal enzymatic activity. In essence, the metabolic reprogramming is part of a signature that creates a highly aggressive and proinflammatory macrophage.

Interestingly, we observed a close correlation of in vitro cathepsin K activity with disease burden in vivo. Higher cathepsin K activity in macrophages predicted more severe disease in RA and CAD, respectively. This finding underlines the functional relevance of such effector cells and gives rise to the model that metabolically reprogrammed macrophages represent a mechanistic link for the acceleration of atherosclerotic disease.

In summary, this study identified a pathogenic GSK3b-dependent pathway as a shared defect in inflammatory macrophages of

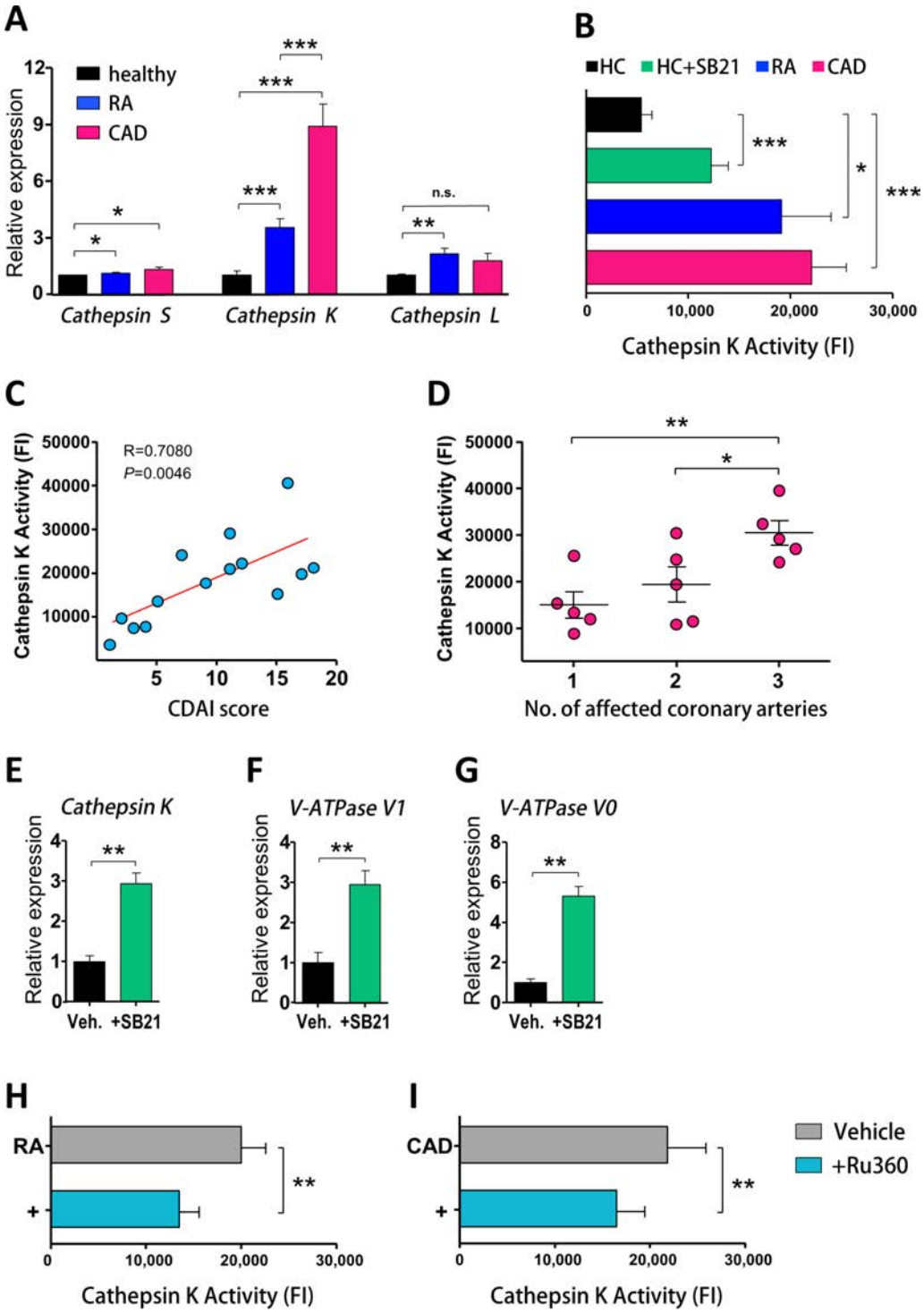

Figure 6 Mitochondrial activity and mitochondria-associated membrane function drive macrophage cathepsin K production. (A) Gene expression measured by RT-PCR in LPS/IFN-γ-stimulated macrophages (n=6 in each group). Results are fold change compared with controls. (B) Cathepsin K enzyme activity measured in activated macrophages (n=8 each group). Healthy macrophages were treated with SB216763 (10 µM) as indicated or vehicle. (C) Correlation of in vitro cathepsin K activity with disease activity (CDAI score) of 14 patients with RA. (D) Association of the extent of cardiovascular disease (number of affected coronary arteries) of 15 patients with CAD with cathepsin K activity. (E–G) Gene expression of activated macrophages from six healthy donors, SB216763 (10 µM) or vehicle was added as indicated. (H–I) Cathepsin K activity of RA and CAD macrophages treated with Ru360 (10 µM); results from seven samples in each group. Unpaired t-test (A–B) and paired t-test (B, E–I) were applied. In (C, D), Pearson correlation coefficient was applied. *P<0.05; **P<0.01; ***P<0.001. All bar graphs show mean±SEM. CAD, coronary artery disease; CDAI, Clinical Disease Activity Index; HC, healthy control; FI, fluorescence intensity; RA, rheumatoid arthritis; V-ATPase, vacuolar-type H^+-ATPases.

patients with RA and CAD. Enhanced formation of MAM structures represents a novel molecular mechanism that drives tissue-destructive effector functions in macrophages. Targeting GSK3b, by restoring its activation status, may allow suppressing effector pathways relevant to RA as well as CAD and provide a novel therapeutic approach towards the increased cardiovascular risk of patients with RA.

Contributors MZ planned and performed experiments, was responsible for data analysis. HZ contributed expertise in tissue analysis. REY, RW, YL and LB contributed technical expertise. BBW and JH enrolled patients and JCG and TLA oversaw patient recruitment. CMW and JJG conceived the study, designed experiments and analysed data and CMW, JJG and MZ wrote the manuscript.

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Competing interests None declared.

Patient consent Obtained.

Ethics approval The study was approved by the Institutional Review Board at Stanford University.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Sack U, Stiehl P, Geiler G. Distribution of macrophages in rheumatoid synovial membrane and its association with basic activity. *Rheumatol Int* 1994;13:181–6.
2 van der Wal AC, Becker AE, van der Loos CM, *et al*. Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. *Circulation* 1994;89:36–44.
3 Bories GFP, Leitinger N. Macrophage metabolism in atherosclerosis. *FEBS Lett* 2017;591:3042–60.
4 Moore KJ, Tabas I. Macrophages in the pathogenesis of atherosclerosis. *Cell* 2011;145:341–55.
5 Peters MJ, van Halm VP, Voskuyl AE, *et al*. Does rheumatoid arthritis equal diabetes mellitus as an independent risk factor for cardiovascular disease? A prospective study. *Arthritis Rheum* 2009;61:1571–9.
6 Crowson CS, Liao KP, Davis JM, *et al*. Rheumatoid arthritis and cardiovascular disease. *Am Heart J* 2013;166:622–8.
7 Ristić GG, Lepić T, Glisić B, *et al*. Rheumatoid arthritis is an independent risk factor for increased carotid intima-media thickness: impact of anti-inflammatory treatment. *Rheumatology* 2010;49:1076–81.
8 Warrington KJ, Kent PD, Frye RL, *et al*. Rheumatoid arthritis is an independent risk factor for multi-vessel coronary artery disease: a case control study. *Arthritis Res Ther* 2005;7:R984–91.
9 Maradit-Kremers H, Crowson CS, Nicola PJ, *et al*. Increased unrecognized coronary heart disease and sudden deaths in rheumatoid arthritis: a population-based cohort study. *Arthritis Rheum* 2005;52:402–11.
10 Littlewood-Evans A, Sarret S, Apfel V, *et al*. GPR91 senses extracellular succinate released from inflammatory macrophages and exacerbates rheumatoid arthritis. *J Exp Med* 2016;213:1655–62.
11 Bae S, Kim H, Lee N, *et al*. α-Enolase expressed on the surfaces of monocytes and macrophages induces robust synovial inflammation in rheumatoid arthritis. *J Immunol* 2012;189:365–72.
12 Shirai T, Nazarewicz RR, Wallis BB, *et al*. The glycolytic enzyme PKM2 bridges metabolic and inflammatory dysfunction in coronary artery disease. *J Exp Med* 2016;213:337–54.
13 Watanabe R, Shirai T, Namkoong H, *et al*. Pyruvate controls the checkpoint inhibitor PD-L1 and suppresses T cell immunity. *J Clin Invest* 2017;127:2725–38.
14 Embi N, Rylatt DB, Cohen P. Glycogen synthase kinase-3 from rabbit skeletal muscle. Separation from cyclic-AMP-dependent protein kinase and phosphorylase kinase. *Eur J Biochem* 1980;107:519–27.
15 Sutherland C. What Are the bona fide GSK3 Substrates? *Int J Alzheimers Dis* 2011;2011:505607.
16 Park SH, Park-Min KH, Chen J, *et al*. Tumor necrosis factor induces GSK3 kinase-mediated cross-tolerance to endotoxin in macrophages. *Nat Immunol* 2011;12:607–15.
17 Cross DA, Alessi DR, Cohen P, *et al*. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature* 1995;378:785–9.
18 Beurel E, Michalek SM, Jope RS. Innate and adaptive immune responses regulated by glycogen synthase kinase-3 (GSK3). *Trends Immunol* 2010;31:24–31.
19 Flügel D, Görlach A, Michiels C, *et al*. Glycogen synthase kinase 3 phosphorylates hypoxia-inducible factor 1alpha and mediates its destabilization in a VHL-independent manner. *Mol Cell Biol* 2007;27:3253–65.
20 Nguyen T, Wong R, Wang G, *et al*. Acute inhibition of GSK causes mitochondrial remodeling. *Am J Physiol Heart Circ Physiol* 2012;302:H2439–45.
21 Sieber MH, Thomsen MB, Spradling AC. Electron transport chain remodeling by GSK3 during oogenesis connects nutrient state to reproduction. *Cell* 2016;164:420–32.
22 Jellusova J, Cato MH, Apgar JR, *et al*. Gsk3 is a metabolic checkpoint regulator in B cells. *Nat Immunol* 2017;18:303–12.
23 Denton RM. Regulation of mitochondrial dehydrogenases by calcium ions. *Biochim Biophys Acta* 2009;1787:1309–16.
24 Territo PR, Mootha VK, French SA, *et al*. Ca(2+) activation of heart mitochondrial oxidative phosphorylation: role of the F(0)/F(1)-ATPase. *Am J Physiol Cell Physiol* 2000;278:C423–35.
25 Lewis SC, Uchiyama LF, Nunnari J. ER-mitochondria contacts couple mtDNA synthesis with mitochondrial division in human cells. *Science* 2016;353:aaf5549.
26 Szabadkai G, Bianchi K, Várnai P, *et al*. Chaperone-mediated coupling of endoplasmic reticulum and mitochondrial Ca2+ channels. *J Cell Biol* 2006;175:901–11.
27 Cheng XW, Huang Z, Kuzuya M, *et al*. Cysteine protease cathepsins in atherosclerosis-based vascular disease and its complications. *Hypertension* 2011;58:978–86.
28 Garnero P, Borel O, Byrjalsen I, *et al*. The collagenolytic activity of cathepsin K is unique among mammalian proteinases. *J Biol Chem* 1998;273:32347–52.
29 Kafienah W, Brömme D, Buttle DJ, *et al*. Human cathepsin K cleaves native type I and II collagens at the N-terminal end of the triple helix. *Biochem J* 1998;331(Pt 3):727–32.
30 Zhou R, Yazdi AS, Menu P, *et al*. A role for mitochondria in NLRP3 inflammasome activation. *Nature* 2011;469:221–5.
31 Horner SM, Liu HM, Park HS, *et al*. Mitochondrial-associated endoplasmic reticulum membranes (MAM) form innate immune synapses and are targeted by hepatitis C virus. *Proc Natl Acad Sci U S A* 2011;108:14590–5.
32 Beurel E, Grieco SF, Jope RS. Glycogen synthase kinase-3 (GSK3): regulation, actions, and diseases. *Pharmacol Ther* 2015;148:114–31.
33 Shi GP, Sukhova GK, Grubb A, *et al*. Cystatin C deficiency in human atherosclerosis and aortic aneurysms. *J Clin Invest* 1999;104:1191–7.
34 Sukhova GK, Shi GP, Simon DI, *et al*. Expression of the elastolytic cathepsins S and K in human atheroma and regulation of their production in smooth muscle cells. *J Clin Invest* 1998;102:576–83.
35 Samokhin AO, Wong A, Saftig P, *et al*. Role of cathepsin K in structural changes in brachiocephalic artery during progression of atherosclerosis in apoE-deficient mice. *Atherosclerosis* 2008;200:58–68.

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EXTENDED REPORT

Novel gene variants associated with cardiovascular disease in systemic lupus erythematosus and rheumatoid arthritis

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ABSTRACT

Objectives Patients with systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) have increased risk of cardiovascular disease (CVD). We investigated whether single nucleotide polymorphisms (SNPs) at autoimmunity risk loci were associated with CVD in SLE and RA.

Methods Patients with SLE (n=1045) were genotyped using the 200K Immunochip SNP array (Illumina). The allele frequency was compared between patients with and without different manifestations of CVD. Results were replicated in a second SLE cohort (n=1043) and in an RA cohort (n=824). We analysed publicly available genetic data from general population, performed electrophoretic mobility shift assays and measured cytokine levels and occurrence of antiphospholipid antibodies (aPLs).

Results We identified two new putative risk loci associated with increased risk for CVD in two SLE populations, which remained after adjustment for traditional CVD risk factors. An *IL19* risk allele, rs17581834(T) was associated with stroke/myocardial infarction (MI) in SLE (OR 2.3 (1.5 to 3.4), $P=8.5\times10^{-5}$) and RA (OR 2.8 (1.4 to 5.6), $P=3.8\times10^{-3}$), meta-analysis (OR 2.5 (2.0 to 2.9), $P=3.5\times10^{-7}$), but not in population controls. The *IL19* risk allele affected protein binding, and SLE patients with the risk allele had increased levels of plasma-IL10 (P=0.004) and aPL (P=0.01). An *SRP54-AS1* risk allele, rs799454(G) was associated with stroke/transient ischaemic attack in SLE (OR 1.7 (1.3 to 2.2), $P=2.5\times10^{-5}$) but not in RA. The *SRP54-AS1* risk allele is an expression quantitative trait locus for four genes.

Conclusions The *IL19* risk allele was associated with stroke/MI in SLE and RA, but not in the general population, indicating that shared immune pathways may be involved in the CVD pathogenesis in inflammatory rheumatic diseases.

INTRODUCTION

Patients with systemic lupus erythematosus (SLE) have a 2–10 fold increased risk of cardiovascular disease (CVD) compared with the general population, with the highest relative risk in younger patients and highest absolute risk in older individuals.[1–3] Women with SLE in the age of 35–45 years have a 50-fold increased risk of myocardial infarction (MI) compared with the general population.[4] Death related to active disease and infections have decreased, but mortality related to CVD shows no such decline.[5] Instead, a slight increase in standardised mortality ratio due to vascular diseases has been reported.[6] Today SLE is acknowledged as a unique risk factor for CVD by the American Heart Association.[7]

Traditional risk factors cannot fully explain the increased risk for CVD in SLE, and a number of SLE-related risk factors have been identified, such as antiphospholipid antibodies (aPL) and renal impairment.[8–10] Genetic predisposition is an important risk factor for SLE, and different risk genes are also connected to CVD. We have previously shown that a variant of interferon regulatory factor 8 (*IRF8*)[11] is associated with development of ischaemic heart disease in SLE and that a variant of signal transducer and activator of transcription 4 (*STAT4*) is associated with aPL and ischaemic stroke.[12] Other risk genes shown to be associated with CVD in SLE include mannose-binding lectin, C reactive protein and HLA-DRB1*04/*13.[13–15] Recently, a large international association study identified 24 new SLE risk loci using Immunochip genotype data.[16 17] In the present study, we examined if single nucleotide polymorphisms (SNPs) analysed by the Immunochip were associated with CVD in two large SLE cohorts and investigated possible functional effects of associated gene variants. We also investigated if identified risk gene variants were associated with CVD in patients with rheumatoid arthritis (RA) and in the general population.

PATIENTS AND METHODS

Patients and controls

The discovery cohort included 1045 patients with SLE from rheumatology clinics in Sweden. All patients fulfilled ≥4 American College of Rheumatology (ACR) 1982 criteria for SLE.[18] The replication cohort included 1043 patients with SLE from the University of California, San Francisco (UCSF) Lupus Genetics project.[19] All patients completed an extensive questionnaire, and the SLE diagnosis was confirmed by medical record review according to

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Table 1 Clinical characteristics of the patients with SLE

	Discovery cohort (Sweden)	Replication cohort (UCSF)	P values
Number of patients	1045	1043	
Female	910 (87)	959 (92)	<0.001
Age at diagnosis (year)	36±16	35±14	0.12
Age at study (year)	51±16	44±13	<0.001
PE/DVT	153 (15)	91 (10)	<0.001
MI/angina*	107 (10)	39 (4)	<0.001
Stroke/TIA†	96 (12)	55 (5)	<0.001
Stroke/MI‡	133 (16)	84 (8)	<0.001
Antiphospholipid antibodies§	292(31)	374(41)	<0.001
ACR criteria			
1) Malar rash	576 (55)	424 (41)	<0.001
2) Discoid rash	244 (23)	60 (6)	<0.001
3) Photosensitivity	699 (67)	816 (78)	<0.001
4) Oral ulcers	245 (23)	282 (27)	0.06
5) Arthritis	823 (79)	727 (70)	<0.001
6) Serositis	459 (44)	269 (26)	<0.001
7) Renal disorder	349 (33)	237 (23)	<0.001
8) Neurological disorder	104 (10)	89 (9)	0.26
9) Haematological disorder	630 (60)	638 (61)	0.72
10) Immunological disorder	711 (68)	640 (61)	0.001
11) Positive ANA	1026 (98)	954 (91)	<0.001
SDI¶ median (range)	1 (0–13)	NA	
BILD** median (range)	NA	2 (0–13)	

Data are number (%) or mean±SD.
Categorical variables were compared with χ^2 test and continuous variables by Student's unpaired t-test.
*Data regarding angina were available only for the discovery cohort.
†Missing data for 231 patients in the discovery cohort, data regarding TIA were only available for the discovery cohort.
‡Missing data for 231 patients in the discovery cohort.
§Discovery cohort, at least one positive test for anticardiolipin (IgM or IgG) or anti-β_2 glycoprotein-I (IgG), data available for 952 patients. Replication cohort, at least one positive test for lupus anticoagulant, anticardiolipin (IgM or IgG) or anti-β_2 glycoprotein-I (IgM or IgG), data available for 907 patients.
¶SDI.[47]
**BILD[48] data available for 514 patients.
ACR criteria, American College of Rheumatology classification criteria for SLE (manifestations until end of follow-up);[18] ANA, antinuclear antibodies BILD, Brief Index of Lupus Damage; DVT, deep vein thrombosis; MI, myocardial infarction; NA, data not available; PE, pulmonary embolism; SDI; Systemic Lupus International Collaborating Clinics /ACR damage index for SLE; SLE, systemic lupus erythematosus; TIA, transient ischaemic attack; UCSF, University of California, San Francisco.

the ACR criteria.[18] The SLE patients were all of European decent, and age at diagnosis was similar in both populations, but patients in the replication cohort were younger at follow-up and had less CVD events (table 1). In the discovery cohort, the average age at first stroke/transient ischaemic attack (TIA) was 52 years and at first stroke/MI was 54 years (online supplementary table S1). Information regarding stroke and TIA was available for 814 patients in the SLE discovery cohort and data regarding TIA, and angina was not available for the replication cohort. Patients with RA (n=824) all fulfilled the 1987 RA classification criteria[20] (online supplementary method/table S2). Healthy blood donors (n=2711) were recruited as previously described.[21] Publicly available data from the CARDIoGRAMplusC4D Consortium[22] and the International Stroke Genetics Consortium[23] were used for analyses of CVD in the general population (online supplementary methods). All participants gave their informed consent. Definitions for CVD in SLE are in online supplementary table S3 and in RA in supplementary methods.

Genotyping and quality control

Genotyping of the SLE discovery cohort, SLE replication cohort and the RA cohort was performed using the Illumina Immunochip (for quality control, see supplementary methods).[16 17 24]

Statistical analysis

A logistic regression model with sex and disease duration included as covariates was used to test association between SNPs and CVD in the SLE discovery cohort. For the American–European population sex, disease duration and the first principal component for population stratification were included as covariates. The identification of SNPs associated with CVD in SLE included three steps. First, the four CVD variables were tested for association with the Immunochip SNPs using data from the discovery cohort. Next, the top 100 associated SNPs per variable were tested for association with CVD in the SLE replication cohort, and SNPs not achieving nominal significance (P>0.05) were excluded. Finally, SNPs with meta-analysis P<0.001 were chosen as candidates for functional follow-up. The identified interleukin 19 (*IL19*) and signal recognition particle 54 – antisense 1(*SRP54-AS1*) SNPs were analysed in the RA cohort, including sex and disease duration as covariates. Resulting significant variants were included in a cross-disease meta-analysis of SLE and RA. In this meta-analysis, $P<1.0\times10^{-6}$ adjusting for 48 000 independent SNPs on the Immunochip were considered significant. In other analyses, p values <0.05 were considered significant. For more information on statistical and bioinformatic analyses, see supplementary methods.

Functional analysis

Electrophoretic mobility shift assays (EMSA) were performed using nuclear extract from Jurkat, LCL, K562, HUVEC cell lines and peripheral blood mononuclear cells (PBMCs) from healthy individuals (supplementary methods). Expression quantitative trait locci (QTLs) were analysed using GTEx datasets,[25] and IL19 (R&D Systems), IL10 (Mesoscale Discovery) and aPL levels were measured (supplementary methods).[12]

Carotid ultrasound

Ultrasound scans were performed as previously described.[26]

RESULTS

Identification of risk loci for CVD in SLE

The allele frequencies of 137 213 SNPs were compared between patients with SLE in the discovery cohort with and without CVD. Initially, we asked if there are general risk genes for CVD in SLE and consequently analysed ischaemic stroke and/or MI (stroke/MI) as one variable. Next, we looked for subtype-specific risk gene variants analysing MI and/or angina (MI/angina) and ischaemic stroke and/or TIA (stroke/TIA) separately. Finally, venous thrombosis defined as pulmonary embolism and/or deep vein thrombosis (PE/DVT) was analysed. We identified eight SNPs at three different loci demonstrating an association with CVD in both the discovery cohort and in the replication cohort, all reaching P<0.001 in the meta-analysis (tables 2 and 3). The *IL19* risk alleles were associated with stroke/MI, the *SRP54-AS1* risk alleles, and the IL7 receptor (*IL7R*) risk alleles were associated with stroke/TIA. Functional studies did not indicate the *IL7R* risk allele to be important for CVD development (see

Table 2 SNP variants associated with stroke/MI

			Discovery cohort (Sweden)*			Replication cohort (UCSF)†			Meta-analysis	
SNP	Locus	M/m	MAF	OR (95% CI)	P values	MAF	OR (95% CI)	P values	OR (95% CI)	P values
rs17581834	IL19	C/T	0.09/0.04	2.6 (1.5 to 4.5)	7.0×10^{-4}	0.09/0.05	1.9 (1.05 to 3.6)	3.7×10^{-2}	2.3 (1.5 to 3.4)	8.5×10^{-5}
rs11119598	IL19	A/G	0.09/0.04	2.5 (1.5 to 4.4)	9.1×10^{-4}	0.09/0.05	1.9 (1.03 to 3.6)	4.1×10^{-2}	2.2 (1.5 to 3.3)	1.2×10^{-4}
rs74148801	IL19	C/T	0.09/0.04	2.5 (1.5 to 4.4)	9.1×10^{-4}	0.09/0.05	1.9 (1.03 to 3.6)	4.1×10^{-2}	2.2 (1.5 to 3.3)	1.2×10^{-4}

The three SNPs showing an association with stroke and/or MI in both cohorts and in the meta-analysis.
*133 patients with and 681 without stroke/MI.
†84 patients with and 959 without stroke/MI.
SNPs with P<0.001 in the meta-analysis were forwarded for functional analyses.
M/m, major/minor alleles; MAF, minor allele frequency for cases/controls; MI, myocardial infarction; P, p value unadjusted; SNP, single nucleotide polymorphisms; UCSF, University of California, San Francisco.

supplementary file). No SNPs were associated with MI/angina or PE/DVT (data not shown).

THE *IL19* LOCUS

Three SNPs in the *IL19* gene (rs74148801, rs17581834 and rs11119598) showed an association with stroke/MI in both SLE cohorts and in the meta-analysis (OR 2.3 (1.5 to 3.4), $P=8.5\times10^{-5}$; table 2). All three SNPs are located in intron 1 of the *IL19* gene and are in high linkage disequilibrium (LD) with each other (1000 Genomes, $r^2=1$) (figure 1A). The association between stroke/MI and the *IL19* risk allele remained significant when adjusting for known cardiovascular risk factors in a multivariable regression analysis (OR 2.03 (1.07 to 3.84), $P=3.09\times10^{-2}$; online supplementary table S4). The *IL19* risk allele was not associated with SLE per se (online supplementary table S5), the ACR criteria (online supplementary table S6)[18] or the SLICC-DI (SDI) (OR 1.30 (0.94 to 1.79), P=0.12).[24] There was no association between the *IL19* risk allele and intima-media thickness (IMT) (0.059 vs 0.063 mm, P=0.92) or presence of carotid plaque (27% vs 21%, P=0.47) in a subgroup of patients with SLE (n=202) examined by carotid ultrasound.

To clarify the function of the *IL19* risk allele, we initially performed EMSAs to investigate effects on transcription factor (TF) binding at the locus. Nuclear extract from Jurkat cells stimulated with PMA/ionomycin or PBMC stimulated with interferon-α (IFN-α) demonstrated binding of a protein to the reference allele (C) but not the risk/alternative allele (T) (figure 1B,C). Nuclear extract from unstimulated Jurkat cells or PBMCs displayed no differential binding between the two alleles. Next, protein expression of genes located at the *IL19* locus, including *IL19* and *IL10* (figure 1A), were examined. Serum-IL19 was measured in 394 SLE patients, but no significant difference between patients with and without the *IL19* risk allele was observed (24% vs 19%, P=0.41). Plasma-IL10 was measured in 243 patients and patients with the *IL19* risk allele more often had elevated IL10 compared with patients without the *IL19* risk allele (50% vs 22%, P=0.0038). SLE patients with high IL10 more often had elevated levels of anticardiolipin (aCL) IgM antibodies compared with patients with low IL10 (31% vs 14%, P=0.01). Given these results, we measured the levels of aPL in 781 patients with SLE and observed an association between the *IL19* risk allele and elevated levels of aCL IgG (P=0.002), aCL IgM (P=0.002), anti-β_2 glycoprotein-I (anti-β_2GPI) IgG (P=0.0004) and antiprothrombin (n=494) IgG (P=0.04) antibodies. Furthermore, the *IL19* risk allele showed an association with positive lupus anticoagulant (LA; n=311) test (P=0.03).

The Signal recognition particle 54 – antisense 1 locus

Three SNPs (rs799454, rs1712349 and rs712308) located in the *SRP54-AS1* gene and in high LD with each other ($r^2=1$) (figure 2A) were associated with stroke/TIA (table 3) with an OR of 1.7 (1.3 to 2.2), $P=2.5\times10^{-5}$ in the SLE meta-analysis. When including the *SRP54-AS1* risk allele together with traditional cardiovascular risk factors, the association remained (OR 1.65 (1.15 to 2.37), $P=6.10\times10^{-3}$; online supplementary table S7).The *SRP54-AS1* risk allele was not associated with SLE (online supplementary table S5), the ACR criteria, levels of aPL, IMT or carotid plaque (data not shown, all P>0.05).

EMSA analysis using nuclear cell extract from Jurkat, LCL, K562 and PBMCs showed protein binding to the *SRP54-AS1* risk locus but no distinct allele specific difference (data not shown). According to chromatin immunoprecipitation with DNA sequencing (ChIP-Seq) data from the ENCODE project,

Table 3 SNP variants associated with stroke/TIA

			Discovery cohort (Sweden)*			Replication cohort (UCSF)†			Meta-analysis	
SNP	Locus	M/m	MAF	OR (95% CI)	P values	MAF	OR (95% CI)	P values	OR (95% CI)	P values
rs799454	SRP54-AS1	A/G	0.57/0.40	1.8 (1.3 to 2.4)	2.2×10^{-4}	0.50/0.40	1.6 (1.03 to 2.3)	3.4×10^{-2}	1.7 (1.3 to 2.2)	2.5×10^{-5}
rs1712349	SRP54-AS1	C/T	0.56/0.40	1.8 (1.3 to 2.4)	2.2×10^{-4}	0.50/0.40	1.6 (1.03 to 2.3)	3.6×10^{-2}	1.7 (1.3 to 2.2)	2.5×10^{-5}
rs712308	SRP54-AS1	C/T	0.56/0.40	1.8 (1.3 to 2.4)	2.2×10^{-4}	0.50/0.40	1.5 (1.02 to 2.3)	4.2×10^{-2}	1.7 (1.3 to 2.2)	3.0×10^{-5}
rs11567698	IL7R	G/T	0.18/0.11	1.8 (1.2 to 2.8)	3.4×10^{-3}	0.18/0.10	1.9 (1.1 to 3.1)	1.6×10^{-2}	1.9 (1.3 to 2.5)	1.5×10^{-4}
rs11567714	IL7R	C/T	0.18/0.11	1.8 (1.2 to 2.8)	3.4×10^{-3}	0.17/0.10	1.9 (1.1 to 3.4)	3.3×10^{-2}	1.9 (1.3 to 2.6)	2.8×10^{-4}

The five SNPs showing an association with stroke and/or TIA in both cohorts.
*96 patients with and 718 without stroke/TIA.
†55 patients with and 988 without stroke; no data regarding TIA in the replication cohort.
SNPs with P<0.001 in the meta-analysis were forwarded for functional analyses.
M/m, major/minor alleles; MAF, minor allele frequency for cases / controls ; P, P value unadjusted; SNP, single nucleotide polymorphisms; TIA, transient ischaemic attack; UCSF, University of California, San Fransisco.

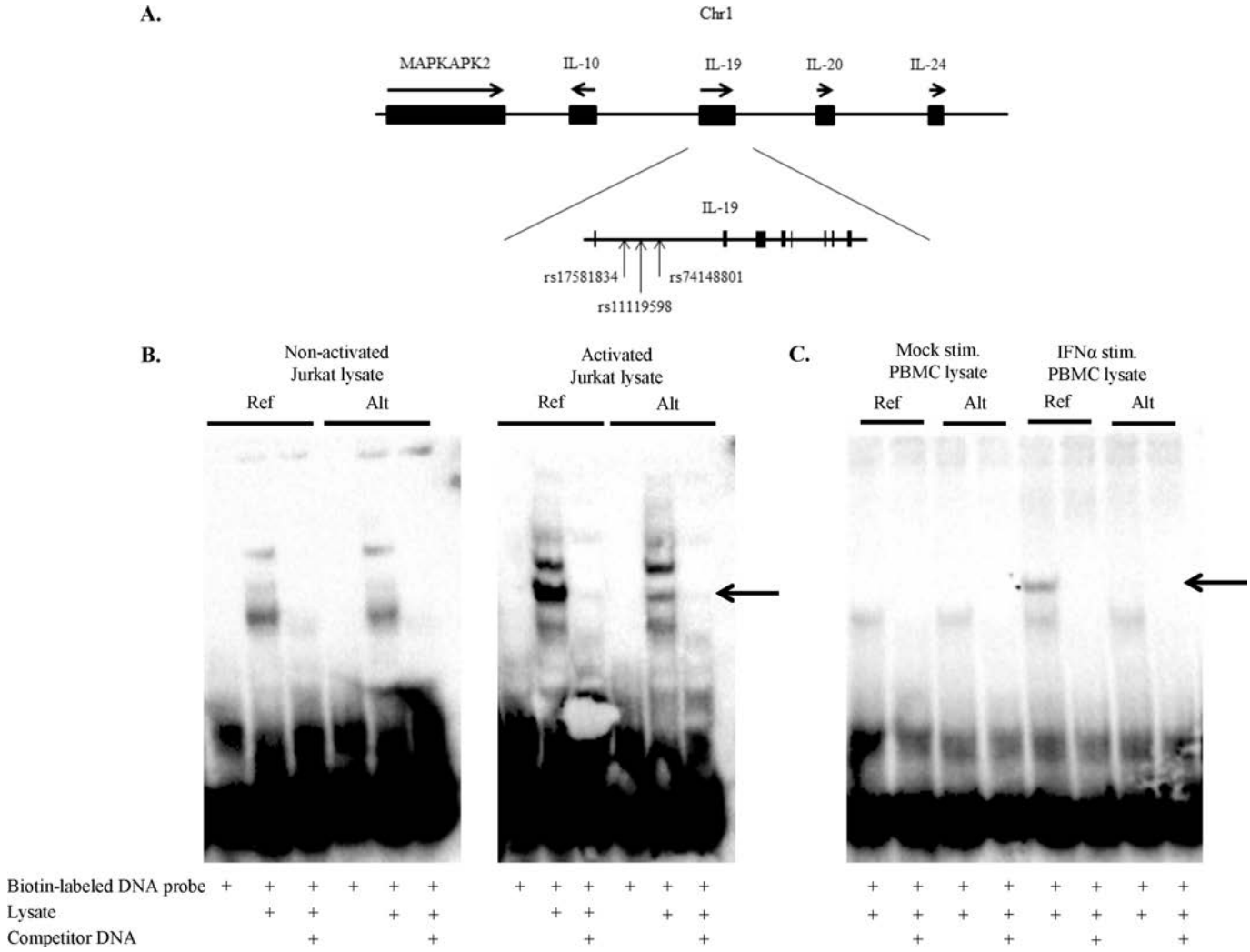

Figure 1 Differential binding to the *IL19* risk allele of a protein produced by PBMCs stimulated with IFN-α and Jurkat cells stimulated with PMA/ionomycin. (A) Schematic picture of the *IL19* gene region on chromosome 1 with the exons shown as vertical bars and the position of the SNPs are indicated by arrows, dbSNP Build 147, release 108. The SNPs rs17581834, rs11119598 and rs74148801 are associated with stroke/MI in SLE. (B) EMSA of the *IL19* locus, rs74148801 using nuclear extract from non-stimulated Jurkat (non-activated) cells and Jurkat cells stimulated with PMA/ionomycin (activated). (C) EMSA of the *IL19* locus, rs74148801 using nuclear extract from non-stimulated PBMCs (Mock stim.) and PBMCs stimulated by IFN-α for 20 hours (IFNα stim.). Arrows point at positions of potential differential binding. Biotin-labelled DNA probe is probes of reference and alternative alleles. Lysate is nuclear extract. Competitor DNA is unlabelled DNA probes as competitors in 100-fold excess. Ref.=reference allele (C); Alt.=alternative allele (T). dbSNP, The Single Nucleotide Polymorphism Database; EMSA; electrophoretic mobility shift assay; IFN, interferon; IL, interleukin; MI, myocardial infarction; PMA, phorbol 12-myristate 13-acetate; PBMCs, peripheral mononuclear cells; SLE, systemic lupus erythematosus; SNPs, single nucleotide polymorphisms.

Signal transducer and activator of transcription 1 (STAT1) binds to the *SRP54-AS1* locus. As STAT1 is crucial for IFN-α signalling, we tested recombinant STAT1 in the EMSA and showed that STAT1 is one of the proteins binding at the *SRP54-AS1* risk locus, with a lower affinity to the risk allele (figure 2B). According to GTEx data, the three SNPs at the *SRP54-AS1* locus affect the expression of signal recognition particle 54 (S54), protein phosphatase 2 regulatory subunit B gamma (PPP2R3C), SRP54-antisense 1 (SRP54-AS1) and family with sequence similarity 177 member A1 (FAM177A1) in multiple tissues (online supplementary figure 1A–D).[25] These genes are all located close to the *SRP54-AS1* risk locus (figure 2A). In addition, the SNP rs712306, in high LD (r^2=0.7), with the *SRP54-AS1* risk allele showed differential expression of FAM177A1 in the artery wall ($P=3.9\times10^{-6}$) (GTEx, online supplementary figure 1E).[25]

The *IL-19* and the *SRP54-AS1* loci in RA and in the general population

In order to investigate if the six identified CVD risk variants were confined to patients with SLE, or could constitute a risk factor for patients with other inflammatory rheumatic diseases, we compared the allele frequencies of the risk gene variants in RA patients with, and without, CVD (table 4). Results show that all 3 *IL19* gene variants were associated with stroke/MI in RA and that allele frequencies of the risk gene variants in patients with and without stroke/MI were similar in RA and SLE (table 4). In addition, the *IL19* risk alleles showed association with stroke/TIA in RA (OR 2.8 (1.4 to 5.6), $P=3.8\times10^{-3}$). The SRP54-AS1 risk allele did not show an association with CVD in RA (data not shown). When including patients with SLE and RA in a meta-analysis, the association with stroke/MI

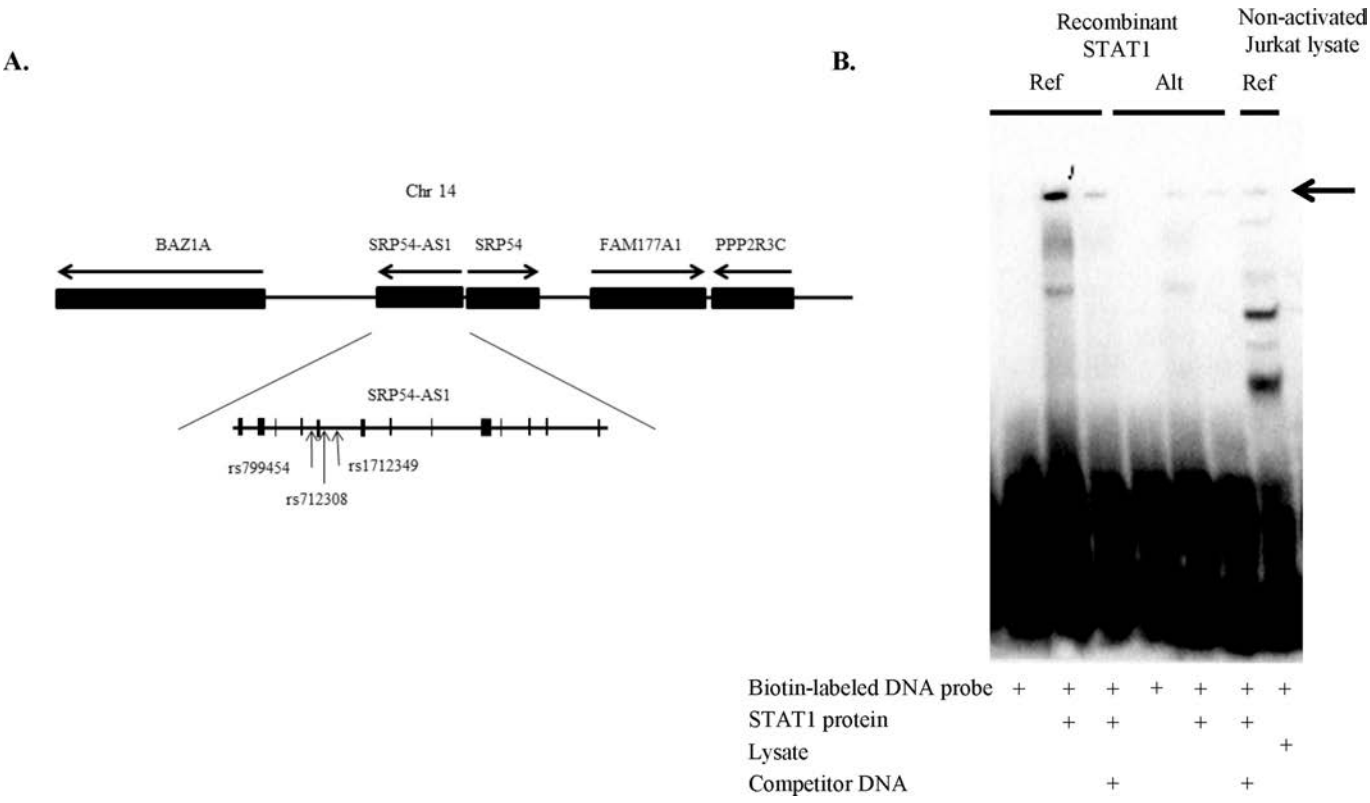

Figure 2 Using recombinant protein, STAT1 is demonstrated to be one of the transcription factors binding at the *SRP54-AS1* locus. (A) Schematic picture of the *SRP54-AS1* gene region on chromosome 14. The exons are shown as vertical bars, and the position of the SNPs are indicated by arrows, dbSNP Build 147, release 108. The SNPs rs799454, rs1712349 and rs712308 are associated with stroke/TIA. (B) EMSA analysis of the *SRP54-AS1* locus, rs1712349. Arrow points at position of potential differential binding of STAT1. Biotin-labelled DNA probe are probes of reference and alternative alleles. STAT1 protein is recombinant STAT1 protein. Lysate is nuclear extract from non-activated Jurkat cells. Competitor DNA is unlabelled DNA probes as competitors in 100-fold excess. Ref.=reference allele (C); Alt.=alternative allele (T). dbSNP, The Short Genetic Variations database; EMSA; electrophoretic mobility shift assay; SNP, single nucleotide polymorphisms; SRP54-AS1, signal recognition particle 54 – antisense 1; STAT1, signal transducer and activator of transcription 1; TIA, transient ischaemic attack.

remained (OR 2.45 (1.96 to 2.94), $P=3.5\times10^{-7}$, after Bonferroni adjustment for 48 000 independent SNPs[16] on the Immunochip $P_{adjusted}=0.017$). Publicly available data of individuals in the general population showed no association between the *IL19* and *SRP54-AS1* risk variants and ischaemic stroke or coronary artery disease (see supplementary results).

DISCUSSION

The objective of the present study was to investigate possible associations between genetic variation and CVD in SLE, and we identified two new risk loci, namely *IL19* and *SRP54-AS1*. The *IL19* risk locus is located in an interleukin gene cluster coding for several cytokines, including IL19 and IL10. Several SNPs in this region have previously been associated with SLE[27 28] and CVD in both the general population[29 30] and in a small SLE study of 52 individuals.[31] However, none of these earlier identified SNPs are in high LD with the SNPs at the *IL19* risk locus identified here, and none of the previously described SNPs were associated with CVD in our dataset. The observation that our identified risk gene variants were not associated with CVD in the general population suggests that there exist at least partly different underlying mechanisms behind CVD in SLE and the general population. The clinical picture for patients with and without the *IL19* risk allele did not differ regarding the ACR criteria[18] or the SDI.[24] Thus, the higher frequency of strokes and MIs in the group with the risk allele is not caused by a more severe SLE disease. Furthermore, when adjusting for known CVD risk factors, the *IL19* risk allele association remained. Thus, our results suggest that the *IL19* risk allele is an independent risk factor for stroke and MI in SLE patients with European decent. The observation that the *IL19* risk allele also was associated with stroke/MI in patients with RA, but not in the general population, furthermore suggest that the gene variants identified could be confined to patients with an inflammatory rheumatic disease, or at least SLE and RA. However, this assumption needs to be confirmed in studies with patients with other diagnoses.

The *IL19* risk locus is a putative binding site for several TFs and is located in a region of open chromatin with predicted enhancer function in LCLs and CD4 and CD8-positive T cells.[32 33] In our EMSA analyses, we observed differential binding to the *IL19* risk allele of nuclear extract from PBMCs stimulated with IFN-α and

Table 4 *IL19* SNPs associated with stroke/MI in rheumatoid arthritis

SNP	Locus	M/m	MAF	OR (95% CI)	P values
rs17581834	IL19	C/T	0.12/0.05	2.7 (1.5 to 5.2)	1.1×10^{-3}
rs11119598	IL19	A/G	0.11/0.05	2.5 (1.4 to 4.6)	3.1×10^{-3}
rs74148801	IL19	C/T	0.11/0.05	2.6 (1.4 to 4.8)	2.1×10^{-3}

Logistic regression analysis between the three *IL19* risk variants and stroke/MI in rheumatoid arthritis (genetic data available for 71 RA patients with and 753 without stroke/MI after RA-disease onset).
IL, interleukin; MAF, minor allele frequency for cases/controls; M/m, major/minor alleles; MI, myocardial infarction; P, p value unadjusted; SNP, single nucleotide polymorphism.

activated Jurkat cells. Because extract from unstimulated cells did not show differential binding to the site, cell activation or high levels of IFN-α as observed in SLE seems to be a requirement for the expression of the relevant protein. Publicly available data[34] identified IKAROS family zinc finger 3 (*IKZF3*) as one possible TF with differential binding to the two alleles. IKZF3 is important for regulation of B cell differentiation, and recently a SNP in the promotor region of *IKZF3* was shown to be associated with SLE.[35] Interestingly, expression of *IKZF3* is regulated by *IRF8*,[36] a TF involved in IFN signalling and associated with CVD in SLE.[11 37] In conclusion, our EMSA analyses suggest that the *IL19* risk locus functions as a cell activation-dependent regulatory region and that the *IL19* risk allele abrogates the binding of a TF induced by IFN-α or T cell activation.

To examine if the *IL19* risk allele affects gene expression, we used data from the GTEx project[25] but found no difference in RNA expression. Next, we went on to analyse protein expression of genes located in the same topologically associated domain[38] and measured IL19 and IL10 levels in the patients with SLE. Similar to previously identified SNPs with long-range functional connections,[39] we found that the intronic *IL19* risk allele affects expression of the *IL10* gene, located at a distance of 30 kb from the *IL19* locus. As IL10 is known to stimulate B cells and antibody production,[40] we measured levels of aPL and indeed found elevated levels of both aCL and $a\beta_2$-GPI as well as LA in patients with the *IL19* risk allele. As presence of aPLs is a known risk factor for CVD,[10 41] especially stroke,[12] these results suggest that the *IL19* risk locus exerts a regulatory effect on IL10 expression, enhancing production of prothrombotic aPL by B cells. However, as both aPL and the *IL19* risk allele remained significantly associated with stroke/MI in the multivariable regression analysis other mechanisms are also possible. Recently, it was reported that IL10 enhances IFN-α-mediated endothelial progenitor cell (EPC) dysfunction and that levels of IL10 correlated with EPC function in SLE but not in healthy controls.[42] Thus, there are several mechanisms whereby IL10 could exert a negative effect on the circulatory system in SLE with implications for cardiovascular pathology.

The *SRP54-AS1* locus was found to be associated with stroke/TIA in SLE. This locus was not associated with a more severe SLE disease, aPLs or subclinical atherosclerosis suggesting a novel mechanism of action. Published ChIP-Seq data indicate that the *SRP54-AS1* locus is located in a region with enhancer/promoter function in CD4, CD8, CD19 and CD14 positive cells with several TFs potentially binding at the locus, including STAT1. In fact, we demonstrated that STAT1 display a lower affinity to the alternative allele. This is interesting given the enhanced expression of STAT1 observed in PBMCs of patients with SLE[43] and its role in intracellular signalling following binding of IFN-α to the type I IFN receptor.[44] According to the GTEx database, *FAM177A1*, *SRP54*, *PPP2R3C* and *SRP54-AS1* are differentially expressed in individuals with the *SRP54-AS1* risk allele. Noteworthy, FAM177A1 is differentially expressed in the artery wall and has previously been associated with neurogenetic disorders.[45] Because FAM177A1 has unknown functions, further studies are needed to determine possible mechanisms connecting FAM177A1 and stroke/TIA in SLE.

The strength of the present study is the large number of patients with SLE and RA investigated, the detailed information regarding CVD events available and that cytokine levels and aPLs were measured in a large number of patients. Furthermore, the Immunochip has approximately 48 000 independent SNPs when LD is considered, and a conservative p value threshold for significance of meta-analysis would be 1×10^{-6}.[16] When including both the patients with SLE and RA in the meta-analysis, the statistical analysis reached this level of significance. Limitations include that the patients in the replication cohort were younger at follow-up resulting in fewer CVD events compared with the discovery cohort and that aPL and stroke data were missing for 20% of patients in the discovery cohort. In addition, we did not have data regarding lipid levels for the patients but identified risk genes have no reported function in lipid metabolism.[46]

In summary, we have identified two potential risk genes of importance for development of CVD in SLE and for *IL19* possibly also in RA. SLE patients with the *IL19* risk allele, associated with stroke/MI, more often had high IL10 and elevated levels of aPL. The *SRP54-AS1* risk allele, associated with stroke/TIA in SLE, was found to likely affect expression of a number of genes. Strikingly, both identified loci seem to affect gene regulation, rather than protein structure. We could also show that the *IL19* gene variant is associated with stroke/MI in both SLE and RA but not in the general population, which suggests the existence of shared mechanisms in the development of CVD in patients with inflammatory rheumatic diseases and this assumption deserves further studies.

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Contributors DL, LR, JKS and AA designed the study. JD, SE, M-LE and DL performed the experiments. KJ-U performed the carotid ultrasound examinations. DL, ES, CB, KET, MF, IG, CS, AAB, SR-D, LAC and LR collected the data. AA, KET, LÄ and DL performed the statistical analysis. DL, AA, KET, JKS, LR, ES, JD, A-CS, LAC, SR-D and M-LE analysed the data. DL, JKS, JD and LR wrote the manuscript. All authors approved the final version of the manuscript.

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REFERENCES

1 Urowitz MB, Bookman AA, Koehler BE, *et al*. The bimodal mortality pattern of systemic lupus erythematosus. *Am J Med* 1976;60:221–5.
2 Bengtsson C, Ohman ML, Nived O, *et al*. Cardiovascular event in systemic lupus erythematosus in northern Sweden: incidence and predictors in a 7-year follow-up study. *Lupus* 2012;21:452–9.
3 Schoenfeld SR, Kasturi S, Costenbader KH. The epidemiology of atherosclerotic cardiovascular disease among patients with SLE: a systematic review. *Semin Arthritis Rheum* 2013;43:77–95.
4 Manzi S, Meilahn EN, Rairie JE, *et al*. Age-specific incidence rates of myocardial infarction and angina in women with systemic lupus erythematosus: comparison with the Framingham Study. *Am J Epidemiol* 1997;145:408–15.
5 Björnådal L, Yin L, Granath F, *et al*. Cardiovascular disease a hazard despite improved prognosis in patients with systemic lupus erythematosus: results from a Swedish population based study 1964-95. *J Rheumatol* 2004;31:713–9.
6 Bernatsky S, Boivin JF, Joseph L, *et al*. Mortality in systemic lupus erythematosus. *Arthritis Rheum* 2006;54:2550–7.
7 Mosca L, Benjamin EJ, Berra K, *et al*. Effectiveness-based guidelines for the prevention of cardiovascular disease in women-2011 update: a guideline from the American Heart Association. *J Am Coll Cardiol* 2011;57:1404–23.
8 Gustafsson JT, Svenungsson E. Definitions of and contributions to cardiovascular disease in systemic lupus erythematosus. *Autoimmunity* 2014;47:67–76.
9 Kaplan MJ, Salmon JE. How does interferon-α insult the vasculature? Let me count the ways. *Arthritis Rheum* 2011;63:334–6.
10 Magder LS, Petri M. Incidence of and risk factors for adverse cardiovascular events among patients with systemic lupus erythematosus. *Am J Epidemiol* 2012;176:708–19.
11 Leonard D, Svenungsson E, Sandling JK, *et al*. Coronary heart disease in systemic lupus erythematosus is associated with interferon regulatory factor-8 gene variants. *Circ Cardiovasc Genet* 2013;6:255–63.
12 Svenungsson E, Gustafsson J, Leonard D, *et al*. A STAT4 risk allele is associated with ischaemic cerebrovascular events and anti-phospholipid antibodies in systemic lupus erythematosus. *Ann Rheum Dis* 2010;69:834–40.
13 Ølhenschlaeger T, Garred P, Madsen HO, *et al*. Mannose-binding lectin variant alleles and the risk of arterial thrombosis in systemic lupus erythematosus. *N Engl J Med* 2004;351:260–7.
14 Szalai AJ, Alarcón GS, Calvo-Alén J, *et al*. Systemic lupus erythematosus in a multiethnic US Cohort (LUMINA). XXX: association between C-reactive protein (CRP) gene polymorphisms and vascular events. *Rheumatology* 2005;44:864–8.
15 Lundström E, Gustafsson JT, Jönsen A, *et al*. HLA-DRB1*04/*13 alleles are associated with vascular disease and antiphospholipid antibodies in systemic lupus erythematosus. *Ann Rheum Dis* 2013;72.
16 Langefeld CD, Ainsworth HC, Cunninghame Graham DS, *et al*. Transancestral mapping and genetic load in systemic lupus erythematosus. *Nat Commun* 2017;8:16021.
17 Cortes A, Brown MA. Promise and pitfalls of the Immunochip. *Arthritis Res Ther* 2011;13:101.
18 Tan EM, Cohen AS, Fries JF, *et al*. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982;25:1271–7.
19 Thorburn CM, Prokunina-Olsson L, Sterba KA, *et al*. Association of PDCD1 genetic variation with risk and clinical manifestations of systemic lupus erythematosus in a multiethnic cohort. *Genes Immun* 2007;8:279–87.
20 Arnett FC, Edworthy SM, Bloch DA, *et al*. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315–24.
21 Almlöf JC, Alexsson A, Imgenberg-Kreuz J, *et al*. Novel risk genes for systemic lupus erythematosus predicted by random forest classification. *Sci Rep* 2017;7:6236.
22 CARDIoGRAMplusC4D. CARDIoGRAMplusC4D Consortium. http://www.cardiogramplusc4d.org/data-downloads/ (accessed 22 Dec 2017).
23 International Stroke Genetics Consortium (ISGC). Welcome to the International Stroke Genetics Consortium. http://cerebrovascularportal.org/ (accessed 11 Jan 2018).
24 Eyre S, Bowes J, Diogo D, *et al*. High-density genetic mapping identifies new susceptibility loci for rheumatoid arthritis. *Nat Genet* 2012;44:1336–40.
25 G. TEx Consortium. The Genotype-Tissue Expression (GTEx) project. *Nat Genet* 2013;45:580–5.
26 Gustafsson JT, Herlitz Lindberg M, Gunnarsson I, *et al*. Excess atherosclerosis in systemic lupus erythematosus,-A matter of renal involvement: Case control study of 281 SLE patients and 281 individually matched population controls. *PLoS One* 2017;12:e0174572.
27 Gateva V, Sandling JK, Hom G, *et al*. A large-scale replication study identifies TNIP1, PRDM1, JAZF1, UHRF1BP1 and IL10 as risk loci for systemic lupus erythematosus. *Nat Genet* 2009;41:1228–33.
28 Zhou M, Ding L, Peng H, *et al*. Association of the interleukin-10 gene polymorphism (-1082A/G) with systemic lupus erythematosus: a meta-analysis. *Lupus* 2013;22:128–35.
29 Kumar P, Yadav AK, Misra S, *et al*. Role of Interleukin-10 (-1082A/G) gene polymorphism with the risk of ischemic stroke: a meta-analysis. *Neurol Res* 2016;38:823–30.
30 Wang BJ, Liu J, Geng J, *et al*. Association between three interleukin-10 gene polymorphisms and coronary artery disease risk: a meta-analysis. *Int J Clin Exp Med* 2015;8:17842–55.
31 Fei GZ, Svenungsson E, Frostegård J, *et al*. The A-1087IL-10 allele is associated with cardiovascular disease in SLE. *Atherosclerosis* 2004;177:409–14.
32 ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. *Nature* 2012;489:57–74.
33 Fingerman IM, McDaniel L, Zhang X, *et al*. NCBI Epigenomics: a new public resource for exploring epigenomic data sets. *Nucleic Acids Res* 2011;39:D908–D912.
34 Thomas-Chollier M, Hufton A, Heinig M, *et al*. Transcription factor binding predictions using TRAP for the analysis of ChIP-seq data and regulatory SNPs. *Nat Protoc* 2011;6:1860–9.
35 Lessard CJ, Adrianto I, Ice JA, *et al*. Identification of IRF8, TMEM39A, and IKZF3-ZPBP2 as susceptibility loci for systemic lupus erythematosus in a large-scale multiracial replication study. *Am J Hum Genet* 2012;90:648–60.
36 Ma S, Pathak S, Trinh L, *et al*. Interferon regulatory factors 4 and 8 induce the expression of Ikaros and Aiolos to down-regulate pre-B-cell receptor and promote cell-cycle withdrawal in pre-B-cell development. *Blood* 2008;111:1396–403.
37 Sjöstrand M, Johansson A, Aqrawi L, *et al*. The Expression of BAFF Is Controlled by IRF Transcription Factors. *J Immunol* 2016;196:91–6.
38 *The 3D Genome Browser: a web-based browser for visualizing 3D genome organization and long-range chromatin interactions*. Biorxiv, 2017. http://biorxiv.org/content/early/2017/02/27/112268. (accessed 27 Feb 2017).
39 Smemo S, Tena JJ, Kim KH, *et al*. Obesity-associated variants within FTO form long-range functional connections with IRX3. *Nature* 2014;507:371–5.
40 Llorente L, Zou W, Levy Y, *et al*. Role of interleukin 10 in the B lymphocyte hyperactivity and autoantibody production of human systemic lupus erythematosus. *J Exp Med* 1995;181:839–44.
41 Toloza SM, Uribe AG, McGwin G, *et al*. Systemic lupus erythematosus in a multiethnic US cohort (LUMINA). XXIII. Baseline predictors of vascular events. *Arthritis Rheum* 2004;50:3947–57.
42 Cates AM, Holden VI, Myers EM, *et al*. Interleukin 10 hampers endothelial cell differentiation and enhances the effects of interferon α on lupus endothelial cell progenitors. *Rheumatology* 2015;54:1114–23.
43 Karonitsch T, Feierl E, Steiner CW, *et al*. Activation of the interferon-gamma signaling pathway in systemic lupus erythematosus peripheral blood mononuclear cells. *Arthritis Rheum* 2009;60:1463–71.
44 Rönnblom L. The importance of the type I interferon system in autoimmunity. *Clin Exp Rheumatol* 2016;34:21–4.
45 Alazami AM, Patel N, Shamseldin HE, *et al*. Accelerating novel candidate gene discovery in neurogenetic disorders via whole-exome sequencing of prescreened multiplex consanguineous families. *Cell Rep* 2015;10:148–61.
46 Willer CJ, Schmidt EM, Sengupta S, *et al*. Discovery and refinement of loci associated with lipid levels. *Nat Genet* 2013;45:1274–83.
47 Gladman D, Ginzler E, Goldsmith C, *et al*. The development and initial validation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index for systemic lupus erythematosus. *Arthritis Rheum* 1996;39:363–9.
48 Yazdany J, Trupin L, Gansky SA, *et al*. Brief index of lupus damage: a patient-reported measure of damage in systemic lupus erythematosus. *Arthritis Care Res* 2011;63:1170–7.

OPEN ACCESS

EXTENDED REPORT

The *STAT4* SLE risk allele rs7574865[T] is associated with increased IL-12-induced IFN-γ production in T cells from patients with SLE

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ABSTRACT

Objectives Genetic variants in the transcription factor *STAT4* are associated with increased susceptibility to systemic lupus erythematosus (SLE) and a more severe disease phenotype. This study aimed to clarify how the SLE-associated intronic *STAT4* risk allele rs7574865[T] affects the function of immune cells in SLE.

Methods Peripheral blood mononuclear cells (PBMCs) were isolated from 52 genotyped patients with SLE. Phosphorylation of STAT4 (pSTAT4) and STAT1 (pSTAT1) in response to interferon (IFN)-α, IFN-γ or interleukin (IL)-12, total levels of STAT4, STAT1 and T-bet, and frequency of IFN-γ$^+$ cells on IL-12 stimulation were determined by flow cytometry in subsets of immune cells before and after preactivation of cells with phytohaemagglutinin (PHA) and IL-2. Cellular responses and phenotypes were correlated to *STAT4* risk allele carriership. Janus kinase inhibitors (JAKi) selective for TYK2 (TYK2i) or JAK2 (JAK2i) were evaluated for inhibition of IL-12 or IFN-γ-induced activation of SLE PBMCs.

Results In resting PBMCs, the *STAT4* risk allele was neither associated with total levels of STAT4 or STAT1, nor cytokine-induced pSTAT4 or pSTAT1. Following PHA/IL-2 activation, CD8$^+$ T cells from *STAT4* risk allele carriers displayed increased levels of STAT4 resulting in increased pSTAT4 in response to IL-12 and IFN-α, and an augmented IL-12-induced IFN-γ production in CD8$^+$ and CD4$^+$ T cells. The TYK2i and the JAK2i efficiently blocked IL-12 and IFN-γ-induced activation of PBMCs from *STAT4* risk patients, respectively.

Conclusions T cells from patients with SLE carrying the *STAT4* risk allele rs7574865[T] display an augmented response to IL-12 and IFN-α. This subset of patients may benefit from JAKi treatment.

Systemic lupus erythematosus (SLE) is an autoimmune rheumatic disease with a strong genetic component.[1] Genetic association studies have identified >80 loci associated with an increased susceptibility to SLE.[2] One of the strongest SLE risk loci outside the HLA region is *signal transducer and activator of transcription* (*STAT*)*4*, with the most significantly associated single-nucleotide polymorphisms (SNPs) in *STAT4* being located in the third intron of the gene.[3–5] However, there is a large linkage disequilibrium (LD) block extending towards the 3′ end of the gene and the causal SNP has not been inferred.[6] Since *STAT1* is located adjacent to *STAT4*, it is possible that the risk SNPs found in *STAT4* have a regulatory effect on *STAT1*.

Both *STAT4* and *STAT1* encode transcription factors of importance to the immune system. STAT4, mainly expressed in T cells and NK cells, is required for the inflammatory response following IL-12 receptor (IL-12R) stimulation.[7–9] STAT4 is also involved in the non-canonical signalling pathway of the type I interferon (IFN) receptor (IFNAR).[10 11] On IL-12R engagement, STAT4 is phosphorylated by JAK2 and TYK2, leading to dimerisation and translocation to the nucleus, where it binds DNA and induces the expression of a large number of proinflammatory genes, including *IFNG*.[12] STAT1 is widely expressed and part of the canonical signalling pathways for the type I IFN receptor (IFNAR) and the IFN-γ receptor (IFNGR).[13] Increased expression and activation of STAT1 has been reported in patients with lupus, which support the important role of the IFN system in SLE.[14]

The SLE-associated *STAT4* SNPs are linked to a more severe disease phenotype with an earlier onset of disease, and an increased risk for stroke and nephritis with severe renal insufficiency.[4 15–17] Despite the strong association of *STAT4* risk SNPs with rheumatic diseases and clinical subphenotypes, very little is known about the molecular mechanisms whereby these risk gene variants contribute to autoimmune disease. Previous studies have reported increased expression of *STAT4* mRNA in osteoblasts[4] and peripheral blood mononuclear cells (PBMCs) from healthy individuals[18] and patients with SLE[19] carrying the *STAT4* risk allele. Furthermore, patients with SLE carrying the *STAT4* risk allele have an increased expression of IFN-induced genes in peripheral blood cells despite having a decreased IFN-α activity in sera.[20] In the present study, we set out to define how the *STAT4* risk variant affects cellular functions in different types of immune cells in SLE. Using primary cells from genotyped patients with SLE, we examined STAT4-dependent and STAT1-dependent cellular functions on the single-cell level in unstimulated and in vitro stimulated cells and correlated these to rs7574865, which is one of the strongest SLE-associated SNPs in *STAT4*. Finally, Janus kinase (JAK) inhibitors were evaluated in vitro for their capacity to restore augmented cellular responses in PBMCs from patients with SLE carrying the *STAT4* risk allele.

PATIENTS AND METHODS

Patients and cells

Peripheral blood was sampled from 52 patients with SLE, fulfilling ≥4 of the 1982 American College of Rheumatology (ACR) classification criteria,[21] at the Rheumatology clinic, Uppsala University Hospital. Disease activity and organ damage were measured with the SLE Disease Activity Index 2000 (SLEDAI-2K)[22] and the Systemic Lupus International Collaborating Clinics/ACR Damage Index,[23] respectively. Patients were genotyped with the Immunochip or the Infinium OmniExpressExome-8 V.1.4 chip (Illumina) and stratified by rs7574865 (NC_000002.12:g.191099907T>G). PBMCs were prepared by Ficoll density-gradient centrifugation and viability frozen in liquid nitrogen. In some experiments, PBMCs were preactivated with 1.5% phytohaemagglutinin (PHA; Life Technologies) and 2.5 ng/mL IL-2 (Miltenyi) for 72 hours. After washing with phosphate buffered saline, cells were rested 4 hours before re-stimulation. IFN-α and IFN-γ levels in plasma were determined with an in-house immunoassay[24] and the human IFN-γ ELISA MAX Standard kit (Biolegend), respectively.

Phosphorylation of *STAT4* and *STAT1*

PBMCs were stimulated with IFN-α2b (IntronA; Schering-Plough), IFN-γ or IL-12 (both Peprotech) at 37°C. Cells were fixed with 2% PFA, permeabilised with Perm Buffer III (BD Biosciences) and stained for cell surface markers (online supplementary table 1). STAT4 and STAT1 protein levels were determined using rabbit polyclonal anti-STAT4 IgG (C20; Santa Cruz) together with PE-labelled F(ab′)2 donkey anti-rabbit IgG or an anti-STAT1 mAb (1/Stat1; BD Biosciences). The geometric mean fluorescence intensity (G-MFI) of STAT4 and STAT1 was divided by the G-MFI of STAT4/STAT1-negative cells. Phospho-specific antibodies to STAT4 (pY693, 38p-Stat4) and STAT1 (pY701, 4a, both BD Biosciences) were used to assess phosphorylation. Cytokine-induced phosphorylation was determined by subtracting the G-MFI of non-stimulated cells from the G-MFI of stimulated cells. Maximal phosphorylation of STAT4 (pSTAT4) and STAT1 (pSTAT1) was detected after 20 min (online supplementary figure S1A,B). EC_{50} values were determined in resting and PHA/IL-2 preactivated cells (IFN-α 200 U/mL, IFN-γ 0.1 ng/mL and IL-12 1 ng/mL; PHA/IL-2 activated cells IFN-α 500 U/mL and IL-12 5 ng/mL; online supplementary figure S1C,D). Unless otherwise stated, a dose of cytokine corresponding to 10 times the EC_{50} dose was used.

IFN-γ production

PHA/IL-2 preactivated cells were re-stimulated with 5 ng/mL IL-12 for 15 hours or 20 ng/mL phorbol 12-myristate-13-acetate (PMA) and 1 ng/mL of the calcium-ionophore A23187 (both Sigma Aldrich) for 6 hours, in the presence of GolgiPlug (BD Biosciences) the last 12 and 5 hours, respectively. Cells were permeabilised with saponin and stained with an anti-IFN-γ (B27) or an isotype control (MOPC-21, both BD Biosciences) mAb. IL-12-specific production of IFN-γ was determined by subtracting the frequency of IFN-γ^+ cells in unstimulated cells.

T-bet expression

T-bet expression was determined using anti-T-bet (O4-46) mAb and the Foxp3 Transcription Factor Staining Buffer (eBiosciences).

Janus kinase inhibitors

The pan-JAK inhibitor (pan-JAKi) tofacitinib,[25 26] the JAK2 selective inhibitor BMS-911543 (JAK2i)[27] and the TYK2 selective inhibitor compound 35 (TYK2i)[28] (all provided by AstraZeneca) were added to cell cultures at indicated concentrations 20 min prior to cytokine stimulation.

Flow cytometry

Flow cytometry data were collected on a FACSCantoII instrument with FACSDiva software V.7.0 (BD Biosciences). Data were analysed using FlowJo software V.10.0.8 (Tree Star).

Statistics

Inter-experiment normalisation was performed as detailed in online supplementary methods. Comparisons of clinical characteristics were performed with the χ^2 or Kruskal-Wallis test. For comparisons of three genotypes, a linear regression model assuming an additive effect was used. No age effect was observed and consequently age was not included as a covariate. For comparisons of two groups, the Mann-Whitney U test was used. Statistical tests were two-tailed. IC_{50} values for JAK inhibitors were determined with a four-parameter logistic regression model. Statistical calculations were performed with GraphPad Prism V.6.02 or IBM SPSS Statistics V.25.

RESULTS

PHA/IL-2-activated T cells from patients with SLE carrying the *STAT4* risk allele have an increased pSTAT4 in response to IL-12 and IFN-α

To study whether rs7574865[T] (hereafter referred to as the *STAT4* risk allele) is associated with alterations in STAT4-dependent or STAT1-dependent cellular traits, we isolated PBMCs from 52 genotyped patients with SLE. All patients had low disease activity (SLEDAI-2K≤4), and the three groups were clinically well matched (table 1).

Initially, the phosphorylation of STAT4 (pSTAT4) and STAT1 (pSTAT1) was determined in $CD56^{dim}$ NK cells, $CD56^{bright}$ NK cells, B cells, $CD4^+$ T cells, $CD4^-$ T cells and monocytes, before and after IFN-α, IFN-γ or IL-12 stimulation. The *STAT4* risk allele was not correlated to basal levels (data not shown), nor IFN-α, IFN-γ-induced or IL-12-induced pSTAT4 or pSTAT1 in

Table 1 Patient characteristics

	rs7574865			
	G/G*	G/T	T/T	P value†
No of patients	21	22	9	
Women, n (frequency)	19 (90%)	20 (91%)	7 (78%)	0.54
Age, years	47 (40–59)	50 (36–66)	36 (32–45)	0.10
Disease duration, years	18 (10–31)	22 (14–31)	19 (11–26)	0.56
No of ACR criteria	6 (5–7)	6 (5–7)	6 (5–7)	0.60
SLEDAI-2K	2 (1–4)	2 (0–4)	0 (0–3)	0.23
SLICC-DI	0 (0–2)	0 (0–1)	1 (0–3)	0.51
Plasma IFN-α, U/mL	0 (0–1.5)	0.4 (0–1.0)	0 (0–0.9)	0.74

Data are presented as median (IQR) for all parameters except sex, for which the number of women and the frequencies are presented.
*G/G, homozygous protective; G/T, heterozygous; T/T, homozygous risk for rs7574865.
†The χ^2 test was used to assess differences in sex distribution and Kruskal-Wallis test was used for the other parameters.
ACR, American College of Rheumatology; IFN-α, interferon alpha; SLEDAI-2K, Systemic Lupus Erythematosus Disease Activity Index 2000; SLICC-DI, Systemic Lupus International Collaborating Clinics/ACR Damage Index.

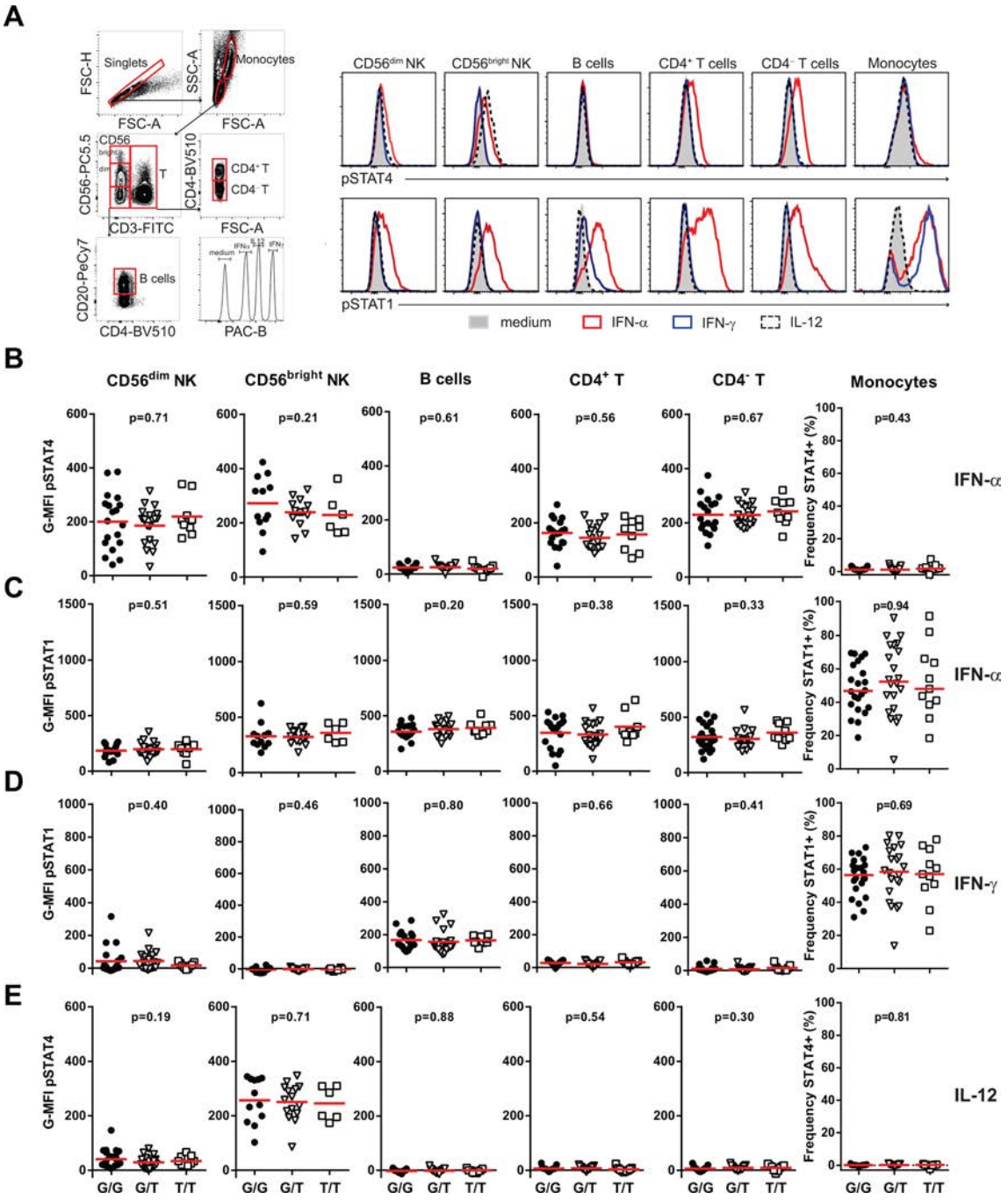

Figure 1 The *STAT4* risk allele does not affect IFN-α, IFN-γ or IL-12-induced phosphorylation of STAT4 or STAT1 in resting peripheral blood mononuclear cells (PBMCs). PBMCs from patients with systemic lupus erythematosus (SLE) were stimulated with 2000 U/mL IFN-α (A–C), 1 ng/mL IFN-γ (A, D) or 10 ng/mL IL-12 (A, E) for 20 min and phosphorylation of STAT4 (pSTAT4) and STAT1 (pSTAT1) was determined in indicated cell population, by flow cytometry. (A) Flow cytometry plots with gating strategy from one representative donor. (B–E) Cumulative data from 19 homozygous protective (G/G, black circles), 21 heterozygous (G/T, open triangles) and 9 homozygous risk (T/T, open squares) *STAT4* patients with SLE. For monocytes, frequencies of $pSTAT4^+$ and $pSTAT1^+$ cells are shown because of the bimodal distributions. Horizontal red bars indicate the mean value. Due to <100 acquired events, data from $CD56^{bright}$ NK cells and B cells are from 12 G/G, 16 G/T and 6 T/T and 17 G/G, 21 G/T and 9 T/T individuals, respectively.

any cell type studied (figure 1A–E). Furthermore, total protein levels of STAT4 and STAT1, and the IFNAR and IFNGR expression were not correlated to the *STAT4* risk allele (online supplementary figure S2).

To further study IL-12 responsiveness in T cells, IL-12R expression was induced by pre-stimulation of cells with PHA and IL-2 for 72 hours.[29] Following re-stimulation with IL-12, an increased pSTAT4 was evident in $CD8^+$ T cells from *STAT4* risk allele carriers (P=0.003, figure 2A,B). This effect was confined to the $CD45RA^+CD57^-$ naïve and the $CD45RA^-CD57^-$ memory $CD8^+$ T cells (P=0.02 and P=0.006, respectively, figure 2C) and the effect was risk allele dosage–dependent with heterozygous *STAT4* risk allele patients displaying intermediate levels of pSTAT4. A slightly increased pSTAT4 was also seen in response to IL-12 in the $CD45RA^-CD57^-$ memory $CD4^+$ T cells (P=0.07, figure 2C). Importantly, current medication (figure 2D), plasma

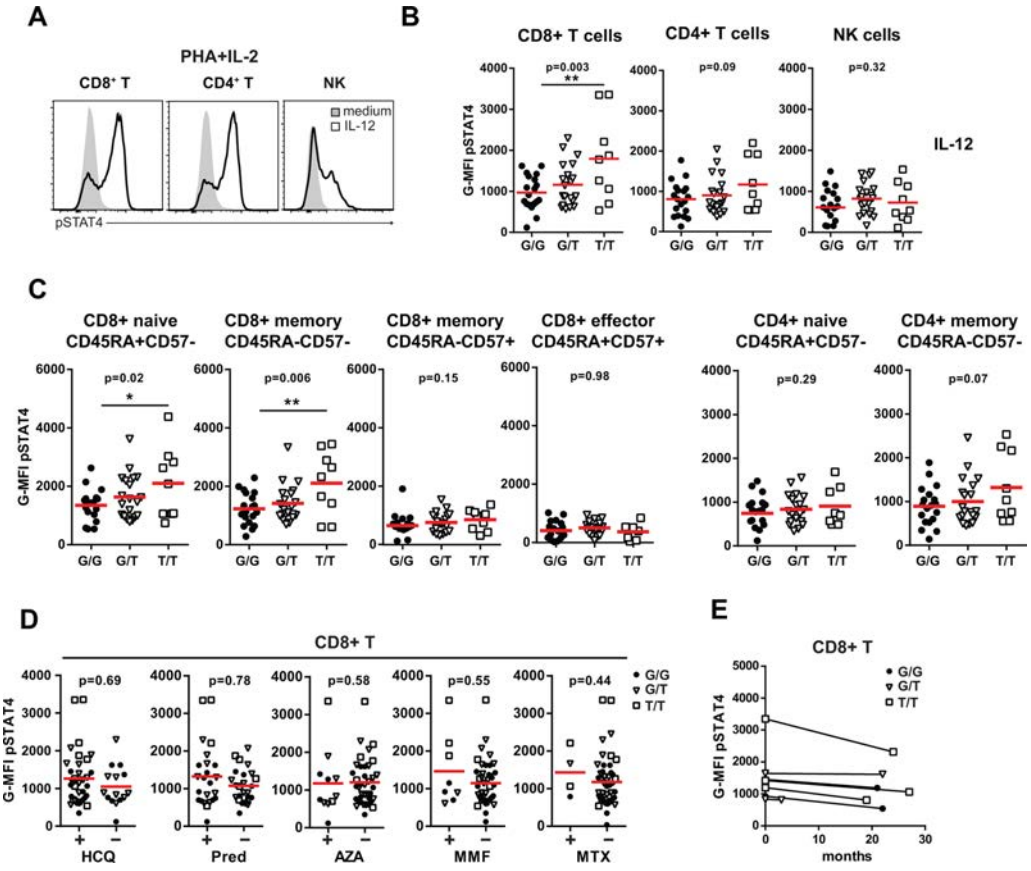

Figure 2 PHA/IL-2 preactivated T cells from patients with systemic lupus erythematosus (SLE) carrying the *STAT4* risk allele have an increased phosphorylation of STAT4 in response to IL-12. PHA/IL-2 pre-activated peripheral blood mononuclear cells were stimulated with 50 ng/mL IL-12 for 20 min. Phosphorylation of STAT4 (pSTAT4) was determined in indicated cell populations using flow cytometry. (A) Histograms from one representative donor. (B–D) Cumulative data from 19 homozygous protective (G/G, black circles), 20 heterozygous (G/T, open triangles) and 9 homozygous risk (T/T, open squares) *STAT4* patients with SLE. Due to <100 acquired events, data from $CD8^+$ effector and $CD4^+$ naïve T cells in (C) are from 17 G/G, 19 G/T and 8 T/T patients with SLE. (D) pSTAT4 in $CD8^+$ T cells from patients stratified for current medication with hydroxychloroquine (HCQ), prednisolone (Pred), azathioprine (AZA), mycophenolate mofetil (MMF) or methotrexate (MTX). (E) pSTAT4 in $CD8^+$ T cells from seven patients sampled twice at indicated time intervals. Horizontal red bars indicate the mean value. *P<0.05 and **P<0.01.

levels of IFN-α (P=0.66) or disease activity (P=0.37) were not associated with the IL-12-response, demonstrating that the increased pSTAT4 in these *STAT4* risk allele carriers was not secondary to disease or therapy. Furthermore, analysis of cells sampled almost 2 years apart showed that the IL-12-induced pSTAT4 in $CD8^+$ T cells was a relatively stable phenotype (figure 2E).

In PHA/IL-2-preactivated PBMCs, slightly increased pSTAT4 and pSTAT1 was also seen in IFN-α-stimulated $CD8^+$ T cells (P=0.04 and P=0.10) from *STAT4* risk allele carriers, but not in $CD4^+$ T cells (P=0.36 and P=0.22) or NK cells (P=0.19 and 0.34, online supplementary figure S3).

In conclusion, PHA/IL-2 preactivated $CD8^+$ T cells from patients with SLE carrying the *STAT4* risk allele have an increased response to IL-12 and IFN-α. Since the strongest effect was seen in IL-12-stimulated cells, we focused our further studies on the IL-12 response.

T cells from patients with SLE carrying the *STAT4* risk allele have increased levels of STAT4 after PHA/IL-2 activation

The increased IL-12-induced pSTAT4 may reflect increased levels of STAT4 or increased IL-12R expression. Analysis of total levels of STAT4 in PHA/IL-2 preactivated $CD45RA^-CD57^-$ memory $CD8^+$ T cells showed a strong correlation with IL-12-induced pSTAT4 (P=0.002, figure 3A). In contrast to resting T cells, PHA/IL-2 preactivated cells from *STAT4* risk allele carriers had increased levels of STAT4 in the $CD45RA^+CD57^-$ naïve (P=0.003) and the $CD45RA^-CD57^-$ memory (P=0.01) $CD8^+$ T cells and slightly increased levels in $CD45RA^+CD57^-$ naïve (P=0.06) and $CD45RA^-CD57^-$ memory (P=0.09) $CD4^+$ T cells (figure 3B).

In terms of IL-12R expression, there were no associations between IL12RB2 expression and IL-12-induced pSTAT4 (figure 3C) or between levels of IL12RB2 and *STAT4* genotype (figure 3D).

Thus, patients with *STAT4* risk allele have an augmented induction of STAT4 protein following PHA/IL-2 stimulation, which may possibly explain the increased response to IL-12.

T cells from patients with SLE carrying the *STAT4* risk allele have an increased production of IFN-γ in response to IL-12

As IL-12-induced IFN-γ production is mediated via STAT4 activation, we next analysed whether the *STAT4* risk allele was associated with increased IFN-γ production. pSTAT4 levels in IL-12-stimulated $CD8^+$ and $CD4^+$ T cells were associated with the frequency of IFN-γ^+ cells (P=0.007 and P=0.04, respectively), and consistent with the phosphorylation data, $CD45RO^+CD57^-$ memory $CD8^+$ T cells from risk allele carriers had a higher frequency of IFN-γ^+ cells (P=0.03, figure 4A). Increased IFN-γ production was also observed

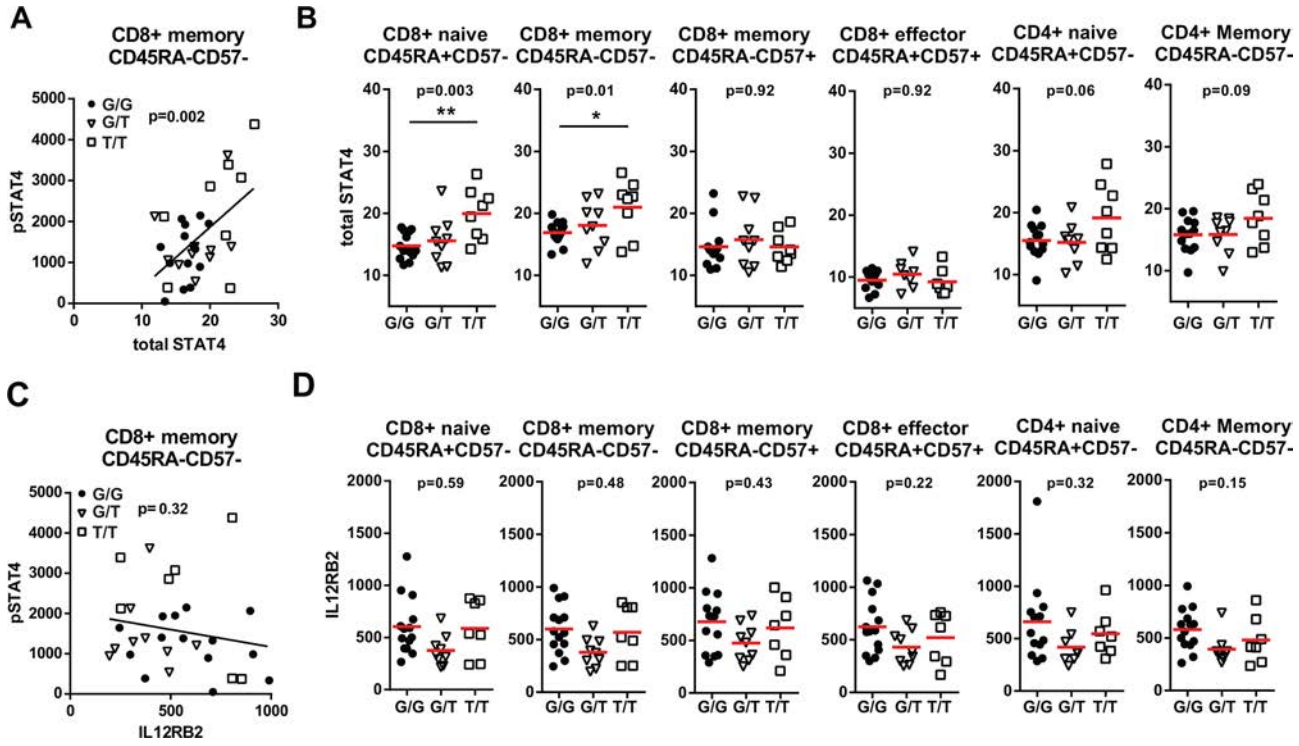

Figure 3 T cells from *STAT4* risk patients with systemic lupus erythematosus (SLE) have increased levels of STAT4 following PHA/IL-2 stimulation. Total protein levels of STAT4 and expression of the IL-12 receptor specific subunit IL12RB2 was determined in PHA/IL-2 preactivated cells in indicated cell populations by flow cytometry. (A, C) Correlation of total levels of STAT4 (A) or IL12RB2 (C) expression with IL-12-induced phosphorylation of STAT4 (pSTAT4) in $CD8^+$ memory T cells. (B, D) Total levels of STAT4 (B) and expression of IL12RB2 (D) in indicated cell populations stratified by *STAT4* genotype. Data from 14 homozygous protective (GG, black circles), 9 heterozygous (G/T, open triangles) and 8 homozygous risk (TT, open squares) *STAT4* patients with SLE. Due to <100 acquired events, data from $CD8^+$ effector T cells are from 12 G/G, 8 G/T and 7 T/T patients with SLE. Horizontal red bars indicate the mean value. *P<0.05 and **P<0.01.

in the $CD45RO^+CD57^-$ memory $CD4^+$ T cells (P=0.06). A general increased IFN-γ production in T cells from *STAT4* risk individuals was excluded by the fact that PMA-induced IFN-γ production was not increased (figure 4B). Despite a risk-allele dosage-dependent numerical increase in IFN-γ plasma levels, this difference was not statistically significant (P=0.32, figure 4C).

The *STAT4* risk allele did not appear to affect Th1 differentiation as neither the frequency of IFN-γ^+ (figure 4B) nor T-bet$^+$ (figure 4D) $CD4^+$ T cells correlated with *STAT4* genotype.

Thus, T cells from patients with SLE carrying the *STAT4* risk allele have an augmented IL-12-induced production of IFN-γ.

JAK inhibitors block the IL-12 response in *STAT4* risk patients with SLE

To investigate the possibility of pharmacological modulation of the IL-12/IFN-γ axis in *STAT4* risk patients, we compared the effect of the pan-JAKi tofacitinib, with two JAKis selectively targeting JAK2 or TYK2. Dose titrations of the JAKis in IL-12, IFN-α or IFN-γ-stimulated cells from healthy individuals revealed that the TYK2i was most selective for IL-12 blockade (figure 5A,B). As expected, the TYK2i also blocked IFN-α-induced activation of cells, whereas the JAK2i blocked IFN-γ-induced activation and the pan-JAKi blocked both IFN-γ-induced and IFN-α-induced activation of cells. Importantly, cells from SLE patients carrying the *STAT4* risk allele were equally sensitive to TYK2i-mediated inhibition of IL-12-induced pSTAT4 and IFN-γ production as cells from non-risk carriers and healthy donors (figure 5C,D). Likewise, the JAK2i and pan-JAKi efficiently blocked IFN-γ signalling in patients with SLE carrying the *STAT4* risk allele (figure 5E). Collectively, these data demonstrate the feasibility to target the IL-12/IFN-γ axis with JAKis in *STAT4* risk allele carriers.

DISCUSSION

Here, we report the first evidence of how an intronic SNP in the major SLE risk locus *STAT4* affects the function of immune cells from patients with SLE in a cell-type-specific and context-dependent manner. We specifically selected patients in remission, or with low disease activity, to avoid effects on the cells due to a prominent ongoing in vivo immune activation. Initially, we analysed pSTAT4 and pSTAT1 in primary immune cells from patients with SLE following IFN-α, IFN-γ and IL-12 stimulation, three cytokines elevated in sera from patients with SLE.[30–32] In resting PBMCs, there was no association of *STAT4* genotype with IFN-α, IFN-γ or IL-12-induced pSTAT4 or pSTAT1. However, re-stimulation of PHA/IL-2 preactivated cells with IL-12 or IFN-α induced a risk-allele dosage-dependent increased pSTAT4 in $CD8^+$ T cells.

The increased IL-12-induced pSTAT4 in $CD8^+$ T cells from *STAT4* risk individuals was accompanied by an augmented IFN-γ production, mainly confined to the $CD45RO^+CD57^-$ memory subset. Increased IFN-γ production was also seen in IL-12-stimulated $CD4^+$ T cells from *STAT4* risk individuals. The augmented IFN-γ production in $CD4^+$ T cells was not due to an increased frequency of Th1 cells as neither the frequency of IFN-γ^+ $CD4^+$ T cells following PMA stimulation nor T-bet expression were increased in *STAT4* risk allele carriers.

STAT4 risk individuals had a numerical increase in their plasma concentrations of IFN-γ, but there was a large inter-individual variation and the difference was not statistically

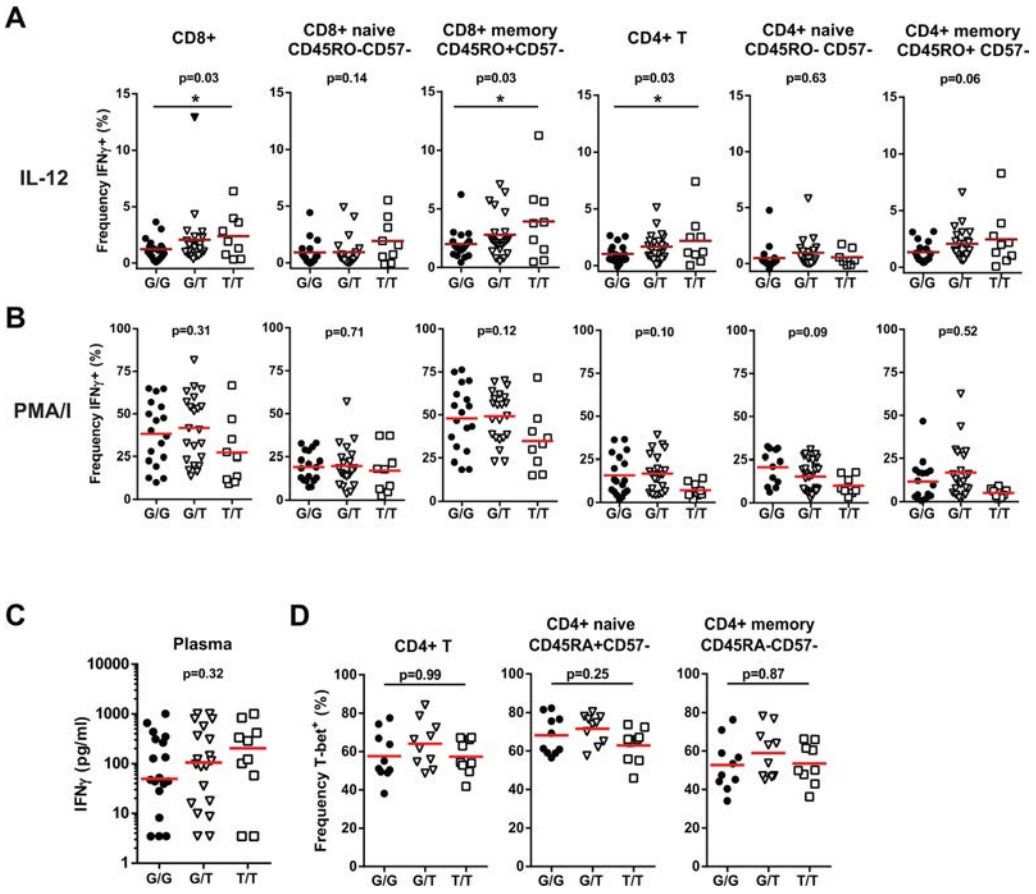

Figure 4 PHA/IL-2 preactivated T cells from *STAT4* risk patients with systemic lupus erythematosus (SLE) have an increased IFN-γ production in response to IL-12. (A, B) PHA/IL-2 activated peripheral blood mononuclear cells were re-stimulated with 5 ng/mL IL-12 for 15 hours (A) or 20 ng/mL PMA together with 1 ng/mL A23187 for 6 hours (B). The frequency of IFN-γ^+ cells was determined in $CD8^+$ and $CD4^+$ T cells and subsets thereof using flow cytometry. Data from 18 *STAT4* homozygous protective (G/G, black circles), 21 heterozygous (G/T, open triangles) and 9 homozygous risk (T/T, open squares) patients with SLE. Data of $CD8^+$ T cells from one G/T individual (indicated as a filled triangle in A) was considered an outlier and was excluded from the statistical analysis of $CD8^+$ T cells and subsets thereof. Due to <100 acquired events, data from one G/G and two G/T from the $CD8^+$ naïve T cells, one G/T and two T/T from the $CD4^+$ naïve T cells, and one G/G from the $CD4^+$ memory T cells were excluded. (C) The concentration of IFN-γ was determined in plasma from 19 G/G, 19 G/T and 10 T/T patients with SLE. Measurements below the detection limit (7 pg/mL, n=7) were assigned a value of half the detection limit. Values above the detection limit (1000 pg/mL, n=4) were assigned a value of 1000 pg/mL. (D) The frequencies of T-bet$^+$ $CD4^+$ T cells in PHA/IL-2 preactivated cells were determined in 12 G/G, 11 G/T and 9 T/T patients with SLE. (A–D) Horizontal red bars indicate the mean value. *P<0.05.

significant. This finding is perhaps not unexpected given that several factors may influence the IFN-γ production in vivo and that the observed effect of the risk allele was both cell-type specific and context dependent.

In line with the previously proposed augmented IFNAR sensitivity,[20] *STAT4* risk allele carriers also displayed increased pSTAT1 in $CD8^+$ naïve T cells in response to IFN-α.

Mechanistically, we found that *STAT4* risk allele carriers had higher total levels of STAT4 in $CD8^+$ and $CD4^+$ T cells following PHA/IL-2 stimulation. Given the intronic nature of rs7574865, it is conceivable that the risk SNP may enhance *STAT4* mRNA expression on PHA/IL-2 stimulation, possibly by altering a transcription factor binding site for a transcription factor preferentially expressed at certain stages of T-cell differentiation. The low numbers of cells available from the patients with SLE and the need to isolate subsets of $CD8^+$ T cells to study mRNA expression have so far precluded studies on the mRNA level.

IFN-γ and IL-12 are closely connected to the pathogenesis of SLE. IFN-γ is a proinflammatory cytokine, central for activation of B cells and macrophages. In murine models of lupus, *Ifng* or *Ifngr* deficiency prevents kidney damage and ameliorates disease.[33–35] In patients with SLE, elevated levels of IFN-γ and IFN-γ-induced chemokines precede the accrual of serum IFN-α activity, autoantibodies and clinical lupus disease by several years.[36] Furthermore, mRNA expression of genes induced by both IFN-α and IFN-γ, but not IFN-α-specific genes, correlates with renal flares.[37] In terms of IL-12, increased serum levels are associated with the presence and severity of lupus nephritis and glomerular IL-12-positive cells are detected in patients with severe lupus nephritis.[32] Given our finding of increased IL-12-induced IFN-γ production in *STAT4* risk individuals, it is thus tempting to speculate that the increased risk for lupus nephritis in *STAT4* risk patients is driven by an increased production of IFN-γ. A recent phase 1b clinical trial of IFN-γ blockade in patients with SLE with lupus nephritis showed no effect on the proteinuria on the aggregate level of patients.[38] However, proteinuria was normalised in some patients on the individual level. Consequently, it would be interesting to stratify patients based on their *STAT4* genotypes in forthcoming studies.

In the context of aberrant regulation of the IL-12/IFN-γ axis, it is interesting to note that several SLE risk loci encode genes that map to this pathway, for example, *IL12A*,[39] *IL12B*,[40 41]

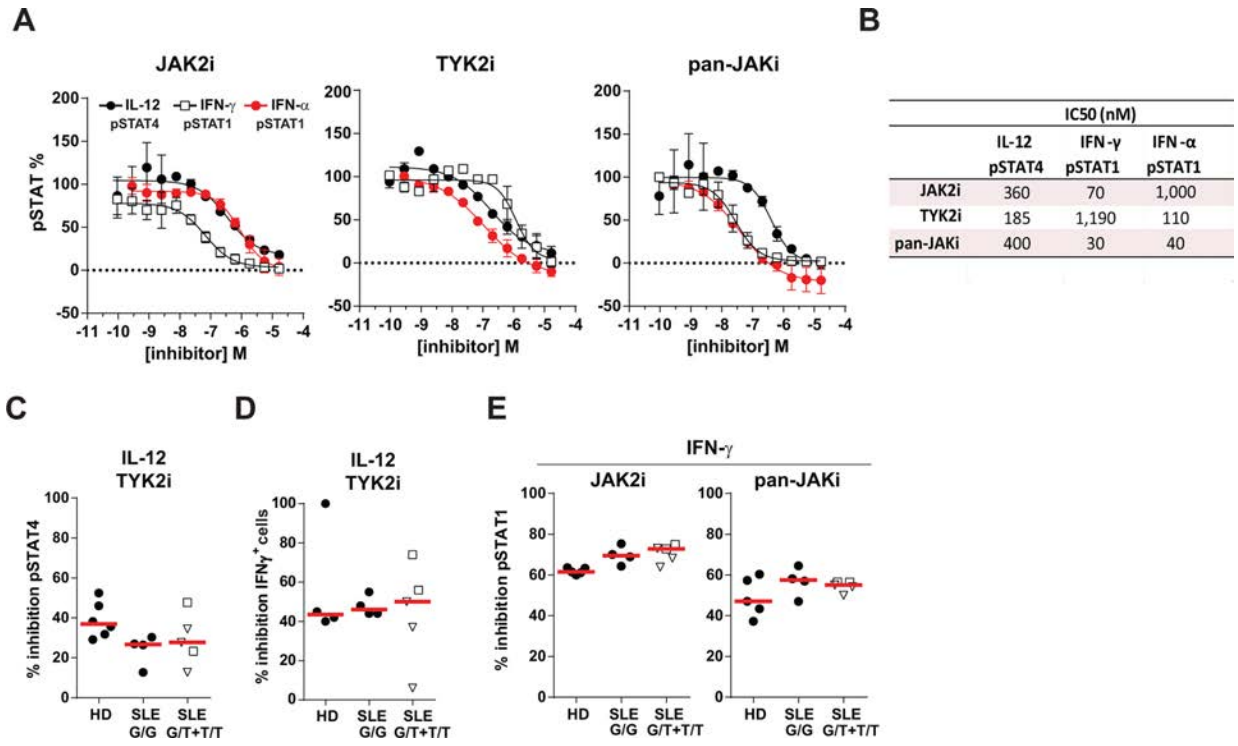

Figure 5 Janus kinase inhibitors block the IL-12 and IFN-γ response in peripheral blood mononuclear cells (PBMCs) from *STAT4* risk patients with systemic lupus erythematosus (SLE). (A) Healthy donor PBMCs were treated with serial dilutions of the JAK2 selective (JAK2i), the TYK2 selective (TYK2i) and the pan-JAK inhibitor (pan-JAKi) before being stimulated with 5 ng/mL IL-12, 0.1 ng/mL IFN-γ or 200 U/mL IFN-α for 20 min. IL-12-stimulated cells were preactivated with PHA/IL-2. Phosphorylation of STAT4 (pSTAT4) and STAT1 (pSTAT1) was determined by flow cytometry in IL-12-stimulated cells and IFN-γ or IFN-α-stimulated cells, respectively. Data (mean±SD of two individuals) from IL-12 and IFN-α-stimulated cells are shown for $CD3^+$ T cells and from IFN-γ-stimulated cells for monocytes. (B) IC_{50} values for each inhibitor. (C–E) PBMCs from healthy donors (HD), patients with SLE homozygous for the protective *STAT4* allele (G/G) and patients with SLE carrying one or two *STAT4* risk alleles (G/T+T/T) were incubated with 200 nM TYK2i (C, D), 70 nm JAK2i or 30 nM pan-JAKi (E). (C, D) Inhibition of IL-12-induced pSTAT4 in $CD8^+$ T cells (C) and reduction in the frequency of IFN-γ^+ memory $CD45RO^+CD57^-$ $CD8^+$ T cells (D). (E) Inhibition of IFN-γ-induced pSTAT1 in monocytes. (C–E) Open triangles and squares denote G/T and T/T patients with SLE, respectively.

IL12RB2,[42] *JAK2*,[43] *TYK2*[44] and *IFNG*.[45] Recently a SNP in *TYK2*, which is protective for SLE, was associated with a decreased response to IL-12R and IFNAR stimulation,[46] suggesting the possibility that genetic dysregulation of the IL-12R response may be a common feature of SLE risk SNPs in locus belonging to the IL-12 pathway. Previous studies have found an additive effect of *STAT4* and *IRF5* risk SNPs,[4 20] and it is conceivable that a combination of several risk gene variants determines the final STAT4 response and contributes to the clinical outcome. However, in this study, we did not have power to investigate possible gene interactions.

The lack of correlation between the IL-12 response and current treatment, and the fact that *STAT4* risk allele carriers have a more severe disease outcome,[4 15–17] may suggest that current standard lupus therapies do not target the pathway affected in *STAT4* risk patients with SLE. The IL-12R signals through JAK2 and TYK2, whereas the IFNGR signalling is mediated through JAK1 and JAK2. Consequently, we compared a JAK2 and a TYK2-selective inhibitor with the pan-JAKi tofacitinib for their ability and selectivity of blocking IL-12, IFN-γ and IFN-α-induced activation of cells. Of the three JAKis evaluated, the TYK2i displayed the highest selectivity for IL-12. Importantly, the TYK2i efficiently blocked IL-12-induced pSTAT4 and IFN-γ production in cells from patients with SLE carrying the *STAT4* risk allele, and the JAK2i blocked IFN-γ-induced activation of SLE *STAT4* risk cells. Since *STAT4* risk patients also had an increased response to IFN-α, and both IFNAR and IL-12R signalling is mediated via TYK2, TYK2 inhibition could be a therapeutic option especially useful in patients carrying the *STAT4* risk allele.

In conclusion, this study establishes a molecular association between the strong genetic SLE risk variant in *STAT4*, rs7574865[T], and an augmented responsiveness to IL-12, which results in increased IFN-γ production in T cells. Furthermore, we demonstrate the feasibility to target the IL-12/IFN-γ axis in patients with SLE carrying the *STAT4* risk allele with JAKis. These findings may have implications for patient stratification in clinical trials and may in the future aid in the selection of drugs for patients carrying the *STAT4* risk allele.

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Contributors NH, YTB, A-CS and LR designed the study; DL and SR collected clinical data; JM and MKN provided reagents; M-LE and DL were responsible for enrolling patients; NH and MJ performed the experiments and analysed data; NH, MJ, YTB and LR interpreted data; NH and LR drafted the manuscript and all authors critically reviewed and approved the final version of the manuscript.

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Competing interests MKN and JM are employed by AstraZeneca.

Patient consent Obtained.

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REFERENCES

1 Kuo CF, Grainge MJ, Valdes AM, *et al*. Familial aggregation of systemic lupus erythematosus and coaggregation of autoimmune diseases in affected families. *JAMA Intern Med* 2015;175:1518–26.
2 Chen L, Morris DL, Vyse TJ. Genetic advances in systemic lupus erythematosus: an update. *Curr Opin Rheumatol* 2017;29:423–33.
3 Remmers EF, Plenge RM, Lee AT, *et al*. STAT4 and the risk of rheumatoid arthritis and systemic lupus erythematosus. *N Engl J Med* 2007;357:977–86.
4 Sigurdsson S, Nordmark G, Garnier S, *et al*. A risk haplotype of STAT4 for systemic lupus erythematosus is over-expressed, correlates with anti-dsDNA and shows additive effects with two risk alleles of IRF5. *Hum Mol Genet* 2008;17:2868–76.
5 Namjou B, Sestak AL, Armstrong DL, *et al*. High-density genotyping of STAT4 reveals multiple haplotypic associations with systemic lupus erythematosus in different racial groups. *Arthritis Rheum* 2009;60:1085–95.
6 Raj P, Rai E, Song R, *et al*. Regulatory polymorphisms modulate the expression of HLA class II molecules and promote autoimmunity. *Elife* 2016;5:e12089.
7 Jacobson NG, Szabo SJ, Weber-Nordt RM, *et al*. Interleukin 12 signaling in T helper type 1 (Th1) cells involves tyrosine phosphorylation of signal transducer and activator of transcription (Stat)3 and Stat4. *J Exp Med* 1995;181:1755–62.
8 Bacon CM, Petricoin EF, Ortaldo JR, *et al*. Interleukin 12 induces tyrosine phosphorylation and activation of STAT4 in human lymphocytes. *Proc Natl Acad Sci U S A* 1995;92:7307–11.
9 Thierfelder WE, van Deursen JM, Yamamoto K, *et al*. Requirement for Stat4 in interleukin-12-mediated responses of natural killer and T cells. *Nature* 1996;382:171–4.
10 Cho SS, Bacon CM, Sudarshan C, *et al*. Activation of STAT4 by IL-12 and IFN-alpha: evidence for the involvement of ligand-induced tyrosine and serine phosphorylation. *J Immunol* 1996;157:4781–9.
11 Nguyen KB, Watford WT, Salomon R, *et al*. Critical role for STAT4 activation by type 1 interferons in the interferon-gamma response to viral infection. *Science* 2002;297:2063–6.
12 Watford WT, Hissong BD, Bream JH, *et al*. Signaling by IL-12 and IL-23 and the immunoregulatory roles of STAT4. *Immunol Rev* 2004;202:139–56.
13 Platanias LC. Mechanisms of type-I- and type-II-interferon-mediated signalling. *Nat Rev Immunol* 2005;5:375–86.
14 Karonitsch T, Feierl E, Steiner CW, *et al*. Activation of the interferon-gamma signaling pathway in systemic lupus erythematosus peripheral blood mononuclear cells. *Arthritis Rheum* 2009;60:1463–71.
15 Taylor KE, Remmers EF, Lee AT, *et al*. Specificity of the STAT4 genetic association for severe disease manifestations of systemic lupus erythematosus. *PLoS Genet* 2008;4:e1000084.
16 Svenungsson E, Gustafsson J, Leonard D, *et al*. A STAT4 risk allele is associated with ischaemic cerebrovascular events and anti-phospholipid antibodies in systemic lupus erythematosus. *Ann Rheum Dis* 2010;69:834–40.
17 Bolin K, Sandling JK, Zickert A, *et al*. Association of STAT4 polymorphism with severe renal insufficiency in lupus nephritis. *PLoS One* 2013;8:e84450.
18 Abelson AK, Delgado-Vega AM, Kozyrev SV, *et al*. STAT4 associates with systemic lupus erythematosus through two independent effects that correlate with gene expression and act additively with IRF5 to increase risk. *Ann Rheum Dis* 2009;68:1746–53.
19 Lamana A, López-Santalla M, Castillo-González R, *et al*. The minor allele of rs7574865 in the STAT4 gene is associated with increased mRNA and protein expression. *PLoS One* 2015;10:e0142683.
20 Kariuki SN, Kirou KA, MacDermott EJ, *et al*. Cutting edge: autoimmune disease risk variant of STAT4 confers increased sensitivity to IFN-alpha in lupus patients in vivo. *J Immunol* 2009;182:34–8.
21 Tan EM, Cohen AS, Fries JF, *et al*. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982;25:1271–7.
22 Gladman DD, Ibañez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. *J Rheumatol* 2002;29:288–91.
23 Gladman D, Ginzler E, Goldsmith C, *et al*. The development and initial validation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index for systemic lupus erythematosus. *Arthritis Rheum* 1996;39:363–9.
24 Cederblad B, Blomberg S, Vallin H, *et al*. Patients with systemic lupus erythematosus have reduced numbers of circulating natural interferon-alpha- producing cells. *J Autoimmun* 1998;11:465–70.
25 Changelian PS, Flanagan ME, Ball DJ, *et al*. Prevention of organ allograft rejection by a specific Janus kinase 3 inhibitor. *Science* 2003;302:875–8.
26 Clark JD, Flanagan ME, Telliez JB. Discovery and development of Janus kinase (JAK) inhibitors for inflammatory diseases. *J Med Chem* 2014;57:5023–38.
27 Purandare AV, McDevitt TM, Wan H, *et al*. Characterization of BMS-911543, a functionally selective small-molecule inhibitor of JAK2. *Leukemia* 2012;26:280–8.
28 Liang J, van Abbema A, Balazs M, *et al*. Lead optimization of a 4-aminopyridine benzamide scaffold to identify potent, selective, and orally bioavailable TYK2 inhibitors. *J Med Chem* 2013;56:4521–36.
29 Desai BB, Quinn PM, Wolitzky AG, *et al*. IL-12 receptor. II. Distribution and regulation of receptor expression. *J Immunol* 1992;148:3125–32.
30 Hooks JJ, Moutsopoulos HM, Geis SA, *et al*. Immune interferon in the circulation of patients with autoimmune disease. *N Engl J Med* 1979;301:5–8.
31 Tokano Y, Morimoto S, Kaneko H, *et al*. Levels of IL-12 in the sera of patients with systemic lupus erythematosus (SLE)—relation to Th1- and Th2-derived cytokines. *Clin Exp Immunol* 1999;116:169–73.
32 Tucci M, Lombardi L, Richards HB, *et al*. Overexpression of interleukin-12 and T helper 1 predominance in lupus nephritis. *Clin Exp Immunol* 2008;154:247–54.
33 Balomenos D, Rumold R, Theofilopoulos AN. Interferon-gamma is required for lupus-like disease and lymphoaccumulation in MRL-lpr mice. *J Clin Invest* 1998;101:364–71.
34 Haas C, Ryffel B, Le Hir M. IFN-gamma receptor deletion prevents autoantibody production and glomerulonephritis in lupus-prone (NZB x NZW)F1 mice. *J Immunol* 1998;160:3713–8.
35 Schwarting A, Wada T, Kinoshita K, *et al*. IFN-gamma receptor signaling is essential for the initiation, acceleration, and destruction of autoimmune kidney disease in MRL-Fas(lpr) mice. *J Immunol* 1998;161:494–503.
36 Munroe ME, Lu R, Zhao YD, *et al*. Altered type II interferon precedes autoantibody accrual and elevated type I interferon activity prior to systemic lupus erythematosus classification. *Ann Rheum Dis* 2016;75:2014–21.
37 Chiche L, Jourde-Chiche N, Whalen E, *et al*. Modular transcriptional repertoire analyses of adults with systemic lupus erythematosus reveal distinct type I and type II interferon signatures. *Arthritis Rheumatol* 2014;66:1583–95.
38 Boedigheimer MJ, Martin DA, Amoura Z, *et al*. Safety, pharmacokinetics and pharmacodynamics of AMG 811, an anti-interferon-γ monoclonal antibody, in SLE subjects without or with lupus nephritis. *Lupus Sci Med* 2017;4:e000226.
39 Bentham J, Morris DL, Graham DSC, *et al*. Genetic association analyses implicate aberrant regulation of innate and adaptive immunity genes in the pathogenesis of systemic lupus erythematosus. *Nat Genet* 2015;47:1457–64.
40 Sun C, Molineros JE, Looger LL, *et al*. High-density genotyping of immune-related loci identifies new SLE risk variants in individuals with Asian ancestry. *Nat Genet* 2016;48:323–30.
41 Yu B, Shao Y, Yue X, *et al*. Copy number variations of Interleukin-12B and T-bet are associated with systemic lupus erythematosus. *Rheumatology* 2011;50:1201–5.
42 Gateva V, Sandling JK, Hom G, *et al*. A large-scale replication study identifies TNIP1, PRDM1, JAZF1, UHRF1BP1 and IL10 as risk loci for systemic lupus erythematosus. *Nat Genet* 2009;41:1228–33.
43 Morris DL, Sheng Y, Zhang Y, *et al*. Genome-wide association meta-analysis in Chinese and European individuals identifies ten new loci associated with systemic lupus erythematosus. *Nat Genet* 2016;48:940–6.
44 Sigurdsson S, Nordmark G, Göring HH, *et al*. Polymorphisms in the tyrosine kinase 2 and interferon regulatory factor 5 genes are associated with systemic lupus erythematosus. *Am J Hum Genet* 2005;76:528–37.
45 Kim K, Cho SK, Sestak A, *et al*. Interferon-gamma gene polymorphisms associated with susceptibility to systemic lupus erythematosus. *Ann Rheum Dis* 2010;69:1247–50.
46 Dendrou CA, Cortes A, Shipman L, *et al*. Resolving TYK2 locus genotype-to-phenotype differences in autoimmunity. *Sci Transl Med* 2016;8:363ra149.

EXTENDED REPORT

Identification of *ST3AGL4*, *MFHAS1*, *CSNK2A2* and *CD226* as loci associated with systemic lupus erythematosus (SLE) and evaluation of SLE genetics in drug repositioning

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ABSTRACT

Objectives Systemic lupus erythematosus (SLE) is a prototype autoimmune disease with a strong genetic component in its pathogenesis. Through genome-wide association studies (GWAS), we recently identified 10 novel loci associated with SLE and uncovered a number of suggestive loci requiring further validation. This study aimed to validate those loci in independent cohorts and evaluate the role of SLE genetics in drug repositioning.

Methods We conducted GWAS and replication studies involving 12 280 SLE cases and 18 828 controls, and performed fine-mapping analyses to identify likely causal variants within the newly identified loci. We further scanned drug target databases to evaluate the role of SLE genetics in drug repositioning.

Results We identified three novel loci that surpassed genome-wide significance, including *ST3AGL4* (rs13238909, p_{meta}=4.40E-08), *MFHAS1* (rs2428, p_{meta}=1.17E-08) and *CSNK2A2* (rs2731783, p_{meta}=1.08E-09). We also confirmed the association of *CD226* locus with SLE (rs763361, p_{meta}=2.45E-08). Fine-mapping and functional analyses indicated that the putative causal variants in *CSNK2A2* locus reside in an enhancer and are associated with expression of *CSNK2A2* in B-lymphocytes, suggesting a potential mechanism of association. In addition, we demonstrated that SLE risk genes were more likely to be interacting proteins with targets of approved SLE drugs (OR=2.41, p=1.50E-03) which supports the role of genetic studies to repurpose drugs approved for other diseases for the treatment of SLE.

Conclusion This study identified three novel loci associated with SLE and demonstrated the role of SLE GWAS findings in drug repositioning.

INTRODUCTION

Systemic lupus erythematosus (SLE (MIM 152700)) is a prototype autoimmune disease with a strong genetic component in its pathogenesis. To date, genome-wide association studies (GWAS) have identified more than 80 SLE-associated loci.[1–7] However, these susceptibility loci taken together only explained less than 30% of disease heritability,[6 7] suggesting that there are many more loci to be identified.

The current SLE GWAS were mainly performed in European and East Asian populations.[6] Through combining GWAS from the two ethnicities, we recently identified 10 novel loci associated with SLE and uncovered a number of loci with suggestive association signal.[6] In this study, we carried out replication studies for those suggestive loci in three independent cohorts, involving a total of 6585 SLE cases and 8435 healthy controls of Chinese ancestry.

Although GWAS studies have achieved great success in mapping disease loci, the vast majority of GWAS findings have not impacted clinical practice such as disease prognosis or treatment which is an area worth further exploration.[8] To date, a number of studies have demonstrated potential applications for GWAS findings in drug repositioning and new drug development.[9–12] In this study, we evaluated the role of SLE genetics in drug repositioning by scanning drug target databases and protein interactions between these molecular targets and SLE susceptibility genes. The results suggest a potential application of SLE GWAS findings in drug repositioning.

METHODS

Participants

This study was designed in two stages. The GWAS datasets used in discovery stage were from our previous studies.[6] Samples for the three GWAS datasets were collected from Hong Kong (612 cases and 2193 controls, HK_GWAS); Anhui Province, mainland China (1047 cases and 1205 controls, AH_GWAS) and Europe (4036 cases and 6959 controls, EUR_GWAS). For replication studies, one of the replication cohorts was from Hong Kong (1673 cases and 1457 controls, HK_rep), and the other two independent cohorts were both from Anhui Province in mainland China, AH_rep1 (3575 cases and 5730 controls) and AH_rep2 (1337 cases and 1248 controls). All the cases fulfilled the revised criteria of the American College of Rheumatology for diagnosis of SLE.[13] The corresponding controls for replication cohorts were geographically and ethnically matched with the cases. The studies were approved by the respective institutional review boards, and all subjects gave informed consent.

Quality control in the discovery stage

For each GWAS cohort, we performed prephasing using SHAPEIT[14] and conducted imputation using IMPUTE2[15] with 1000 Genomes Project data as reference (phase I integrated set, March 2012, build 37). Single nucleotide polymorphisms (SNPs) with imputation INFO score <0.9 were filtered out. For each GWAS cohort, we removed SNPs with >5% missing data or with minor allele frequency (MAF) <1%, and subjects with >5% missing data. We then tested for Hardy-Weinberg equilibrium (HWE) in each GWAS dataset and removed SNPs with HWE p<1.00E-04 in the controls. We performed association analysis for each of the three studies separately based on an additive model using SNPTEST.[16] To combine association results from different GWAS datasets, we performed meta-analysis based on the inverse variance-based method from METAL.[17]

Candidate loci selection and genotyping in the replication stage

SNPs with meta p value <5E-05 in the discovery stage were selected for further validation. SNPs within 200 kilobase pair (kbp) of known loci for the disease (±200 kbp of the reported SNP)[6] were excluded. Twenty-eight SNPs were chosen for further validation. We first used Sequenom to genotype the selected SNPs in samples from HK_rep and AH_rep1. Twenty-four SNPs were successfully genotyped with a call rate higher than 90%. SNP rs763361 failed HWE test (p<1.00E-04) in the controls of AH_rep1 cohort. SNPs satisfying the following criteria were selected for the second replication stage using samples from AH_rep2: (1) with association p value <0.1 in either HK_rep or AH_rep1 cohort; (2) the direction of association in HK_rep and AH_rep1 cohorts was the same as that in the GWAS. Five SNPs were selected and replicated in AH_rep2 cohort using TaqMan genotyping method. SNP rs13238909 failed to be replicated in AH_rep2 cohort because of low call rate (<70%).

Fine-mapping and functional analyses for variants at the newly identified loci

We extracted all variants that passed quality control in the newly identified loci, with the boundaries determined by recombination rate <10 cM/Mb.[18] A fine-mapping algorithm, PAINTOR V.3.0,[19 20] was used to calculate the posterior probability of causality for each variant in a given locus, based on a multiethnic model. To refine the model, all annotations on DNase-seq peaks were integrated. The credible sets of variants that are 70% likely to contain the causal variants were defined as putative causal variants. To further evaluate the regulatory role of the likely causal variants, epigenomic annotations, such as data on DNase-seq, H3K27ac and H3K4me1 peaks from immune-related cell lines were collected from Encyclopedia of DNA Elements (ENCODE) and ROADMAP Epigenomics projects.[21 22] Expression quantitative trait loci (eQTL) in lymphoblastoid cell lines (LCLs) as well as in naive and stimulated monocytes were also obtained from previous studies.[23 24] To ask whether associations from GWAS and an eQTL are driven by the same underlying genetic effect, we applied COLOC[25] to calculate the posterior probability of a shared causal effect (PP4) between GWAS data and eQTL data, using default prior probability.

Identification of putative SLE susceptibility genes

We tallied genes in the surrounding regions (±200 kb) of reported risk loci for SLE, excluding the major histocompatibility complex (MHC) region. The reported SLE loci were retrieved from Morris *et al*.[6] On average, about five genes in each locus were mapped. To highlight potential genes that are regulated by risk variants, genes in the susceptibility loci supported by any one of the following biological annotations would be defined as putative risk genes: (a) genes with missense variants also being SLE-associated variants or in high linkage disequilibrium (LD) with them (r^2>0.8); (b) genes whose expression is associated with reported SLE variants in cis, the significant eQTLs were collected from immune-related cell types, including T cells,[26 27] B cells,[28] monocytes,[26 28] LCLs[23 27 29] and whole blood[30]; (c) genes that are differentially expressed or methylated in SLE, these data were collected from previous studies[31–34]; (d) genes that are prioritised by DAPPLE[35] or PrixFixe.[36] The tools selected likely risk genes based on commonality in functional annotations from different associated loci. The putative risk genes would be assigned to the ones that are closest to the reported variants if genes within the loci do not have any biological supports for being risk genes.

Drug repositioning for SLE treatment

To evaluate the role of SLE genetics in drug repositioning, we curated the targets of approved drugs for SLE treatment by scanning two databases, the DrugBank V.4.3[37] and the Therapeutic Target Database V.4.3.02.[38] We used protein–protein interaction (PPI) information from InWeb[39] to evaluate the potential relationships between SLE risk genes and drug targets. The graphics of PPI network were generated using Cytoscape V.3.5.1.[40]

RESULTS

Identification of novel SLE susceptibility loci

We selected 28 loci showing suggestive association signals (p<5E-05) from meta-analysis of the three SLE GWAS datasets on both European and Chinese populations (see the Methods section).[6] We then conducted a replication study for those selected loci in 6585 SLE cases and 8435 healthy controls of Chinese ancestry. The selected variants were first genotyped in HK_rep and AH_rep1 cohorts using Sequenom. After that, five variants were further examined using TaqMan assays in the AH_rep2 cohort (see the Methods section).

We then performed meta-analysis on the data from both GWAS and replication cohorts, involving a total of 12 280 cases and 18 828 controls, and identified four loci surpassed genome-wide significance (table 1): rs13238909 (*ST3AGL4*, p_{meta}=4.40E-08, OR=0.85), rs2428 (*MFHAS1*, p_{meta}=1.17E-08, OR=1.13), rs2731783 (*CSNK2A2*, p_{meta}=1.08E-09, OR=1.12) and rs763361 (*CD226*, p_{meta}=2.45E-08, OR=1.12). Among them, the *CD226* locus (rs763361) was recently reported to be associated with SLE in the Korean population.[7]

Fine-mapping and functional implications of newly identified susceptibility loci

To explore functional implications of the four newly identified susceptibility loci, we first performed fine-mapping using PAINTOR[19 20] and identified 9, 1, 2 and 26 putative causal variants at *ST3AGL4*, *MFHAS1*, *CSNK2A2* and *CD226* loci, respectively (see online supplementary table S1). We then integrated these results with annotations on epigenomic markers and cis-regulatory elements, derived from three major immune-related cells including LCLs, T cells and monocytes. These analyses indicated that the putative causal variants in *CSNK2A2* and *CD226* loci might regulate gene expression in a cell type-specific manner (see online supplementary table S2).

The *CSNK2A2* locus presents an illustrative example. We observed that the replicated variant (rs2731783) in the locus

Table 1 Summary statistics for the four newly identified loci that are associated with SLE

SNP	Gene region	Chr	Position (hg19)	Ref/alt allele	Cohorts*	RAF_cases	RAF_controls	OR	SE	P values	P_het
rs13238909	*ST3AGL4*	7	67076373	A/G	Meta (10 943/17 544)	–	–	0.85	0.03	4.40E-08	0.84
					EUR GWAS (4036/6959)	0.151	0.174	0.86	0.04	1.00E-04	
					HK GWAS (612/2193)	0.031	0.042	0.73	0.18	8.42E-02	
					AH_GWAS (1047/1205)	0.062	0.069	0.89	0.12	3.35E-01	
					HK_rep (1673/1457)	0.029	0.033	0.89	0.14	4.35E-01	
					AH_rep1 (3575/5730)	0.054	0.065	0.82	0.06	2.68E-03	
rs2428	*MFHAS1*	8	8641145	T/C	Meta (10 943/17 544)	–	–	1.13	0.02	1.17E-08	0.04
					EUR GWAS (4036/6959)	0.511	0.477	1.15	0.03	1.67E-06	
					HK GWAS (612/2193)	0.866	0.864	1.02	0.09	8.34E-01	
					AH_GWAS (1047/1205)	0.860	0.874	0.88	0.09	1.56E-01	
					HK_rep (1673/1457)	0.880	0.861	1.18	0.07	3.31E-02	
					AH_rep1 (3575/5730)	0.886	0.868	1.17	0.05	4.43E-04	
rs2731783	*CSNK2A2*	16	58253460	A/G	Meta (12 280/18 828)	–	–	1.12	0.02	1.08E-09	0.16
					EUR GWAS (4036/6959)	0.176	0.153	1.17	0.04	3.68E-05	
					HK GWAS (612/2193)	0.327	0.303	1.12	0.07	1.08E-01	
					AH_GWAS (1047/1205)	0.382	0.345	1.17	0.06	1.10E-02	
					HK_rep (1673/1457)	0.327	0.308	1.09	0.06	1.19E-01	
					AH_rep1 (3575/5730)	0.367	0.353	1.06	0.03	5.67E-02	
					AH_rep2 (1337/1248)	0.411	0.362	1.23	0.06	5.64E-04	
rs763361	*CD226*	18	67531642	T/C	Meta (8705/13 062)	–	–	1.12	0.02	2.45E-08	0.30
					EUR GWAS (4036/6959)	0.501	0.474	1.12	0.03	7.52E-05	
					HK GWAS (612/2193)	0.347	0.334	1.06	0.07	3.90E-01	
					AH_GWAS (1047/1205)	0.365	0.354	1.05	0.06	4.39E-01	
					HK_rep (1673/1457)	0.330	0.299	1.15	0.06	2.49E-02	
					AH_rep2 (1337/1248)	0.360	0.313	1.24	0.06	3.33E-04	

*Number of cases/number of controls for each cohort.
AH, Anhui Province; Chr, chromosome; EUR, Europe; GWAS, genome-wide association studies; HK, Hong Kong; P het, p value for heterogeneity test across different cohorts; RAF, frequency of reference allele; Ref/alt, reference/alternative; rep, replication cohort; SLE, systemic lupus erythematosus.

was a shared signal in European (p=3.68E-05) and Asian GWAS (p=3.01E-03), being the peak association signal on meta-analysis of GWAS data from the two ethnicities (p=3.07E-07, figure 1A). In addition to rs2731783, 80 other variants in this region also showed strong association with SLE (p<1.00E-05). We applied fine-mapping algorithm PAINTOR and identified two likely causal variants (rs2550368 and rs2731741) in the region which were located around 17 kbp upstream of *CSNK2A2* and 35 kbp upstream of *CCDC113* (figure 1B). Both variants are in high LD with rs2731783 (r^2>0.95 in both European and Asian populations).

We further examined epigenomic annotations in this region with the aim of dissecting the functional implications of the putative causal variants. Data on DNase hypersensitive peaks suggest that this region is highly active in LCLs, but not in T cells and monocytes. Consistent with the DNase cell type-specific signature, the two putative casual variants seem to reside in an LCL-specific enhancer that is defined by both H3 lysine 27 acetylation (H3K27ac) and H3 lysine 4 monomethylation (H3K4me1) markers (figure 1C).

We then asked which gene(s) is/are potential target(s) for the putative causal variants through examining correlations between the genotypes and expression levels of nine genes within 200 kbp of the putative causal variants. We found that the risk allele (rs2550368-G) was associated with reduced expression of *CSNK2A2* (Spearman p=5.0E-10) and *CCDC113* (Spearman p=5.1E-06) in LCLs, but not in naïve and stimulated monocytes (figure 1D,E). We further used a Bayesian method[25] to test for colocalisation between SLE GWAS and eQTL signals (see the Methods section). The results showed a high posterior probability for a shared causal effect (PP4=96.1%) between SLE association and *CSNK2A2* expression in LCLs (see online supplementary figure S1), suggesting that the signals for SLE GWAS and eQTL were likely driven by the same causal variant. Compared with *CSNK2A2* expression in LCLs, we observed a lower posterior probability for a shared causal effect (PP4=79.4%) between SLE association and *CCDC113* expression (see online supplementary figure S1). The expression level of *CCDC113* was also significantly lower than that of *CSNK2A2* (paired t-test p<2.2E-16, figure 1E) in LCLs. Taken together, these results suggest that the putative causal variants may regulate expression of *CSNK2A2* through affecting enhancer activities in B lymphocytes.

Evaluation of SLE genetics in drug repositioning

To evaluate the role of SLE genetics in drug repositioning, we identified 78 putative SLE risk genes by integrating multiple annotation sources (see online supplementary table S3) and obtained 30 genes targeted by SLE drugs (see online supplementary table S4). Although only *NFKBIA* was mapped as both an SLE risk gene and a pharmacological target for SLE, 18 of the 78 SLE risk genes (23.1%) were found encoding proteins interacting with SLE drug targets (figure 2A). The proportion is significantly greater than that for genes in general within the PPI network (OR=2.41, χ^2 p=1.50E-03). Instead of using putative SLE risk genes, we repeated the analysis using protein-coding genes within the flanking regions (±200 kbp) of SLE-associated SNPs. The result showed similar trend, but was insignificant (OR=1.32, χ^2 p=0.123), probably due to the inclusion of many unrelated genes in the associated loci.

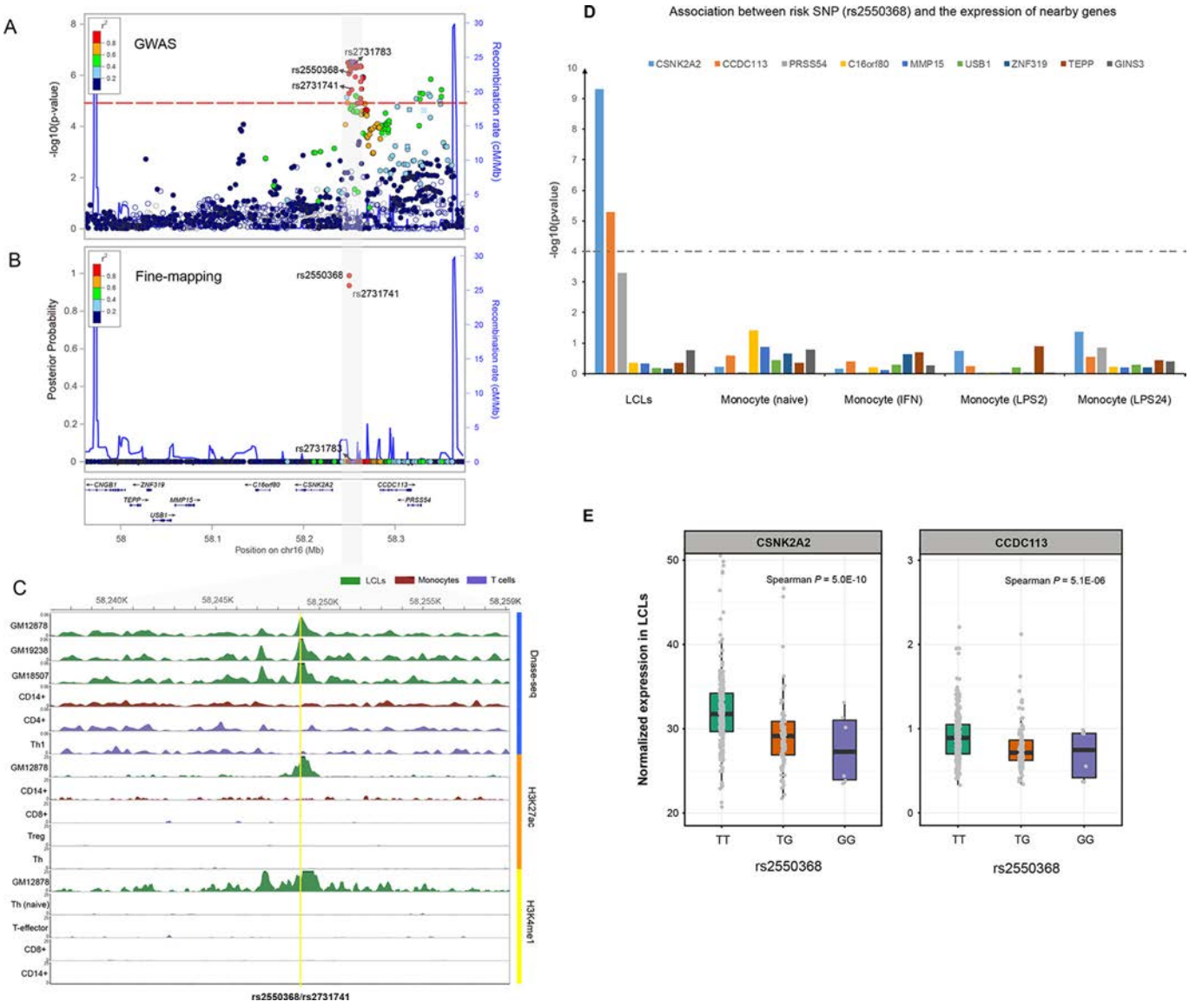

Figure 1 Fine-mapping and functional implications for variants in *CSNK2A2* locus. (A) Regional plot of SLE GWAS associations. (B) Posterior probability of causality for each variant in the region. (C) Overlap between the putative risk variants and functional annotations in LCLs (green), monocytes (red) and T cells (purple). (D) Associations between the risk SNP (rs2550368) and expression of its nearby genes in LCLs, naïve and stimulated monocytes. Y axis indicates –log10(*p values*) for the associations. (E) Expressions of *CSNK2A2* and *CCDC113* in LCLs are associated with the genotype of putative causal variant (rs2550368). GWAS, genome-wide association studies; LCL, lymphoblastoid cell line; SLE, systemic lupus erythematosus.

In contrast, we observed no significant enrichment in interaction between the putative SLE risk genes and targets of drugs not intended for SLE (OR=1.08, χ^2 p=0.849). To further confirm the result, we extended the analyses using drugs intended for unrelated diseases with numbers of targets comparable with that of SLE drugs, namely glaucoma and type 2 diabetes (T2D) which included 25 and 28 drug targets, respectively (see online supplementary tables S5 and S6). We showed that the proportion of putative SLE risk genes interacting with glaucoma drug targets is not significantly different from that expected by chance (OR=1.22, Fisher exact test p=0.682) which was also similar for T2D drugs (OR=1.27, Fisher exact test p=0.562).

DISCUSSION

In this study, we identified three novel loci associated with SLE (*ST3AGL4*, *MFHAS1* and *CSNK2A2*) and also confirmed the association of *CD226* locus with SLE. From the original GWAS datasets, we observed that the statistical power of these newly identified loci was much lower in Asians than that in Europeans. This could be attributed to smaller sample size and lower MAFs (except for rs2731783) in Asian GWAS. For example, the MAF of newly identified SNP rs13238909 in Asians (<7%) is lower than that in Europeans (>15%). We further revisited the data and confirmed that the genotyping quality was good (see online supplementary table S7) and seemed not a factor affecting the association.

We identified putative causal variants at the newly identified loci through fine-mapping algorithms, and further analyses suggested that the putative causal variants in *CSNK2A2* locus may regulate expression of *CSNK2A2* through affecting enhancer activities in B-lymphocytes. *CSNK2A2* encodes an enzyme (CK2) that phosphorylates a number of immune-related TFs and JAK2, playing an important role in JAK-STAT activation.[41] Although the data from public domains are consistent

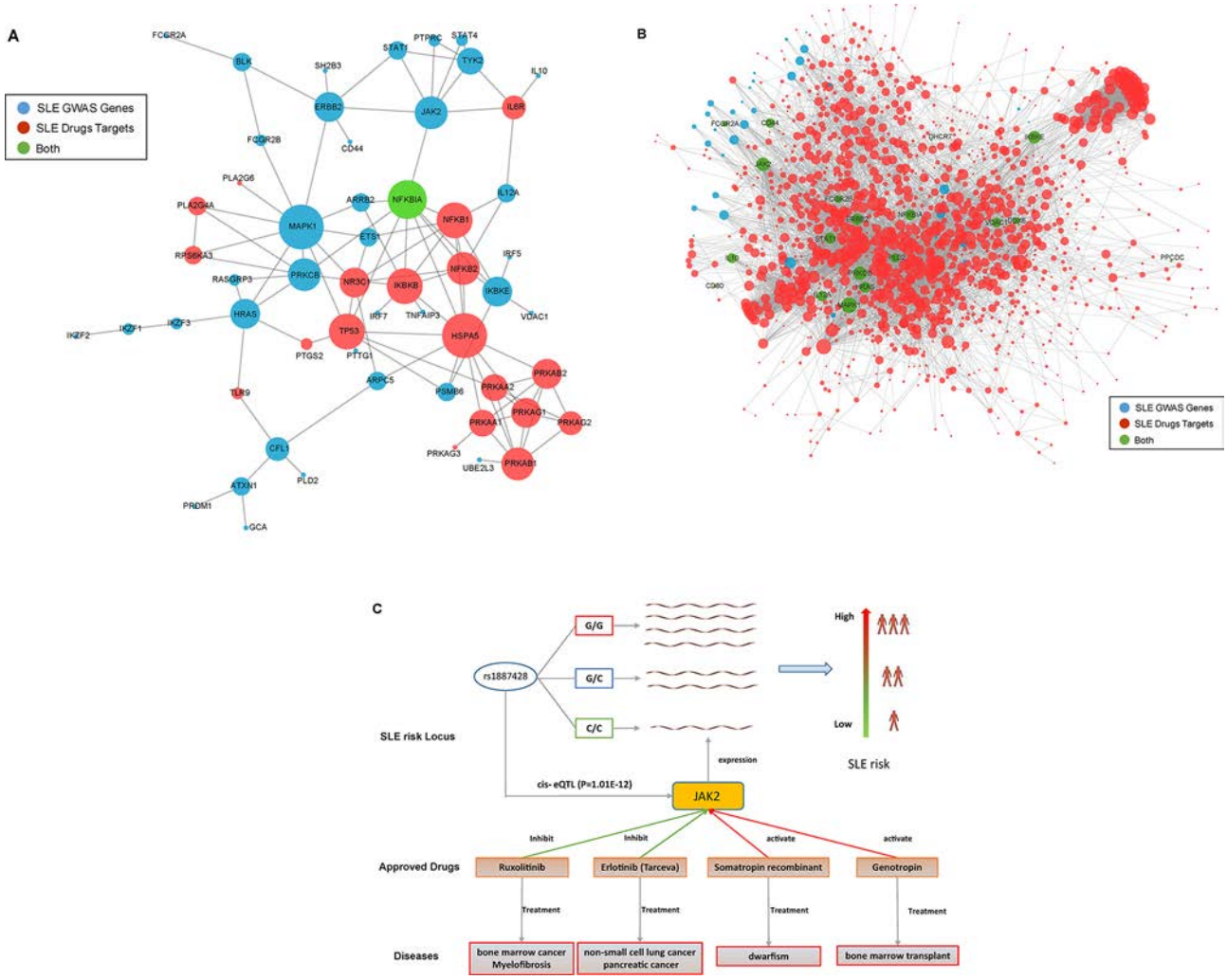

Figure 2 Drug repositioning for the systemic lupus erythematosus (SLE) treatment (A) protein–protein interaction (PPI) network of putative SLE risk genes and genes targeted by SLE drugs. Blue nodes represent SLE risk genes, red nodes indicate genes targeted by SLE drugs. *NFKB1A*, which is considered a risk gene for SLE and a target by SLE drugs, is labelled in green. (B) PPI network of SLE risk genes and genes targeted by all drugs. (C) Illustration of the potential of jakinibs for SLE treatment and the potential side-effect of jak-activators for patients with SLE. GWAS, genome-wide association studies.

here, further molecular assays (eg, CRISPR-Cas9 editing) are needed to confirm the function of these variants and the mechanism of the association.

Understanding the connection between genetic findings and pharmaceutical targets will facilitate translation of GWAS findings to clinical utility in future. Our study demonstrated that SLE risk genes are more likely to be interacting proteins with targets of the approved SLE drugs. We further found 19 SLE risk genes as targets of drugs not intended for SLE (figure 2B), providing a clue to repurposing existing drugs for the SLE treatment. For example, ruxolitinib and erlotinib (Tarceva) are Food and Drug Administration (FDA)-approved drugs indicated for cancers (figure 2C), functioning as *JAK1* and *JAK2* inhibitors (jakinibs). *JAK2* is a risk gene for SLE, encoding a kinase closely involved in JAK/STAT, interferon and cytokine signalling.[42] eQTL data demonstrated that the risk allele (rs1887428-G) was associated with increased expression of *JAK2* (p=1.01E-12).[30] Based on these information, we speculate a potential of jakinibs for the treatment of SLE. Recent studies on mouse model provide certain support for the hypothesis which have demonstrated that ruxolitinib could attenuate cutaneous lupus development in a mouse model.[43] The drug is also now being tested for treatment of other inflammatory diseases (NCT01950780, NCT02809976).[44 45] Similarly, we found an experimental drug, momelotinib (CYT387, NCT01969838) which was designed as an inhibitor for both TBK1/IKBKE and JAK/STAT signalling.[46] Since both *JAK2* and *IKBKE* have been reported as SLE susceptibility loci,[6] we speculate that momelotinib may be an efficacious drug for SLE treatment as it targets more than one SLE-related pathways (see online supplementary figure S2).

In contrast, we speculate that two drugs (somatropin-recombinant and Genotropin), functioning as *JAK2* activators, may accelerate disease progress if given to patients with SLE (figure 2C). A recent survey may support our concern which showed that 12 individuals out of 3745 cases who reported side effects of Genotropin to FDA during the period from 2004 to 2012 developed SLE. The percentage (0.3204%) is nearly nine times greater than any other drugs (0.0362%).[47]

There are a few caveats in these analyses, since both lists of the causal genes for disease associations and targeted genes for treatment options can be incomplete, and inaccurate at times. For example, the ways used to identify SLE risk genes may miss the real causal genes as the real target could be mapped far away from the associated variants.[48] In addition, the validity of drug

repositioning also depends on our knowledge of the targets of existing drugs. Thus, more investigations are necessary to lead to repurposing of existing drugs for SLE treatment, and to gain further insight from genetic findings.

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Contributors WY, YLL, YZ, YS and Y-FW designed the association study. Y-FW and YZ wrote the manuscript. WY and JJS revised the manuscript. YZ selected SNPs for validation. YZ, ZZ, YS, HZ, H-FP, JY, SY, D-QY, XZ and YC designed and performed replication studies. Y-FW designed and performed fine-mapping studies. Y-FW and T-YW designed and performed the study of drug repositioning. DLM and TJV shared systemic lupus erythematosus association data in European populations.

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REFERENCES

1 Harley JB, Alarcón-Riquelme ME, Criswell LA, *et al*. Genome-wide association scan in women with systemic lupus erythematosus identifies susceptibility variants in ITGAM, PXK, KIAA1542 and other loci. *Nat Genet* 2008;40:204–10.
2 Han JW, Zheng HF, Cui Y, *et al*. Genome-wide association study in a Chinese Han population identifies nine new susceptibility loci for systemic lupus erythematosus. *Nat Genet* 2009;41:1234–7.
3 Yang W, Shen N, Ye DQ, *et al*. Genome-wide association study in Asian populations identifies variants in ETS1 and WDFY4 associated with systemic lupus erythematosus. *PLoS Genet* 2010;6:e1000841.
4 Yang W, Tang H, Zhang Y, *et al*. Meta-analysis followed by replication identifies loci in or near CDKN1B, TET3, CD80, DRAM1, and ARID5B as associated with systemic lupus erythematosus in Asians. *Am J Hum Genet* 2013;92:41–51.
5 Bentham J, Morris DL, Graham DSC, *et al*. Genetic association analyses implicate aberrant regulation of innate and adaptive immunity genes in the pathogenesis of systemic lupus erythematosus. *Nat Genet* 2015;47:1457–64.
6 Morris DL, Sheng Y, Zhang Y, *et al*. Genome-wide association meta-analysis in Chinese and European individuals identifies ten new loci associated with systemic lupus erythematosus. *Nat Genet* 2016;48:940–6.
7 Sun C, Molineros JE, Looger LL, *et al*. High-density genotyping of immune-related loci identifies new SLE risk variants in individuals with Asian ancestry. *Nat Genet* 2016;48:323–30.
8 Visscher PM, Brown MA, McCarthy MI, *et al*. Five years of GWAS discovery. *Am J Hum Genet* 2012;90:7–24.
9 Okada Y, Wu D, Trynka G, *et al*. Genetics of rheumatoid arthritis contributes to biology and drug discovery. *Nature* 2014;506:376–81.
10 Visscher PM, Wray NR, Zhang Q, *et al*. 10 Years of GWAS Discovery: Biology, Function, and Translation. *Am J Hum Genet* 2017;101:5–22.
11 Manolio TA. Bringing genome-wide association findings into clinical use. *Nat Rev Genet* 2013;14:549–58.
12 Nelson MR, Tipney H, Painter JL, *et al*. The support of human genetic evidence for approved drug indications. *Nat Genet* 2015;47:856–60.
13 Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997;40:1725.
14 Delaneau O, Marchini J. 1000 Genomes Project Consortium. Integrating sequence and array data to create an improved 1000 Genomes Project haplotype reference panel. *Nat Commun* 2014;5:3934.
15 Howie B, Fuchsberger C, Stephens M, *et al*. Fast and accurate genotype imputation in genome-wide association studies through pre-phasing. *Nat Genet* 2012;44:955–9.
16 Marchini J, Howie B, Myers S, *et al*. A new multipoint method for genome-wide association studies by imputation of genotypes. *Nat Genet* 2007;39:906–13.
17 Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* 2010;26:2190–1.
18 Myers S, Bottolo L, Freeman C, *et al*. A fine-scale map of recombination rates and hotspots across the human genome. *Science* 2005;310:321–4.
19 Kichaev G, Pasaniuc B. Leveraging Functional-Annotation Data in Trans-ethnic Fine-Mapping Studies. *Am J Hum Genet* 2015;97:260–71.
20 Kichaev G, Yang WY, Lindstrom S, *et al*. Integrating functional data to prioritize causal variants in statistical fine-mapping studies. *PLoS Genet* 2014;10:e1004722.
21 Bernstein BE, Birney E. ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. *Nature* 2012;489:57–74.
22 Kundaje A, Meuleman W, Ernst J, *et al*. Integrative analysis of 111 reference human epigenomes. *Nature* 2015;518:317–30.
23 Lappalainen T, Sammeth M, Friedländer MR, *et al*. Transcriptome and genome sequencing uncovers functional variation in humans. *Nature* 2013;501:506–11.
24 Fairfax BP, Humburg P, Makino S, *et al*. Innate immune activity conditions the effect of regulatory variants upon monocyte gene expression. *Science* 2014;343:e1246949.
25 Giambartolomei C, Vukcevic D, Schadt EE, *et al*. Bayesian test for colocalisation between pairs of genetic association studies using summary statistics. *PLoS Genet* 2014;10:e1004383.
26 Raj T, Rothamel K, Mostafavi S, *et al*. Polarization of the effects of autoimmune and neurodegenerative risk alleles in leukocytes. *Science* 2014;344:519–23.
27 Gutierrez-Arcelus M, Lappalainen T, Montgomery SB, *et al*. Passive and active DNA methylation and the interplay with genetic variation in gene regulation. *Elife* 2013;2:e00523.
28 Fairfax BP, Makino S, Radhakrishnan J, *et al*. Genetics of gene expression in primary immune cells identifies cell type-specific master regulators and roles of HLA alleles. *Nat Genet* 2012;44:502–10.
29 Xia K, Shabalin AA, Huang S, *et al*. seeQTL: a searchable database for human eQTLs. *Bioinformatics* 2012;28:451–2.
30 Westra HJ, Peters MJ, Esko T, *et al*. Systematic identification of trans eQTLs as putative drivers of known disease associations. *Nat Genet* 2013;45:1238–43.
31 Ko K, Koldobskaya Y, Rosenzweig E, *et al*. Activation of the Interferon Pathway is Dependent Upon Autoantibodies in African-American SLE Patients, but Not in European-American SLE Patients. *Front Immunol* 2013;4:309.
32 Lee HM, Mima T, Sugino H, *et al*. Interactions among type I and type II interferon, tumor necrosis factor, and beta-estradiol in the regulation of immune response-related gene expressions in systemic lupus erythematosus. *Arthritis Res Ther* 2009;11:R1.
33 Lee HM, Sugino H, Aoki C, *et al*. Underexpression of mitochondrial-DNA encoded ATP synthesis-related genes and DNA repair genes in systemic lupus erythematosus. *Arthritis Res Ther* 2011;13:R63.
34 Absher DM, Li X, Waite LL, *et al*. Genome-wide DNA methylation analysis of systemic lupus erythematosus reveals persistent hypomethylation of interferon genes and compositional changes to CD4+ T-cell populations. *PLoS Genet* 2013;9:e1003678.
35 Rossin EJ, Lage K, Raychaudhuri S, *et al*. Proteins encoded in genomic regions associated with immune-mediated disease physically interact and suggest underlying biology. *PLoS Genet* 2011;7:e1001273.
36 Taşan M, Musso G, Hao T, *et al*. Selecting causal genes from genome-wide association studies via functionally coherent subnetworks. *Nat Methods* 2015;12:154–9.
37 Law V, Knox C, Djoumbou Y, *et al*. DrugBank 4.0: shedding new light on drug metabolism. *Nucleic Acids Res* 2014;42:D1091–7.
38 Zhu F, Shi Z, Qin C, *et al*. Therapeutic target database update 2012: a resource for facilitating target-oriented drug discovery. *Nucleic Acids Res* 2012;40:D1128–36.
39 Lage K, Karlberg EO, Størling ZM, *et al*. A human phenome-interactome network of protein complexes implicated in genetic disorders. *Nat Biotechnol* 2007;25:309–16.
40 Shannon P, Markiel A, Ozier O, *et al*. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* 2003;13:2498–504.
41 Zheng Y, Qin H, Frank SJ, *et al*. A CK2-dependent mechanism for activation of the JAK-STAT signaling pathway. *Blood* 2011;118:156–66.
42 Villarino AV, Kanno Y, O'Shea JJ. Mechanisms and consequences of Jak-STAT signaling in the immune system. *Nat Immunol* 2017;18:374–84.
43 Chan ES, Herlitz LC, Jabbari A. Ruxolitinib Attenuates Cutaneous Lupus Development in a Mouse Lupus Model. *J Invest Dermatol* 2015;135:1912–5.
44 Mesa RA. Ruxolitinib, a selective JAK1 and JAK2 inhibitor for the treatment of myeloproliferative neoplasms and psoriasis. *IDrugs* 2010;13:394–403.

45 Mackay-Wiggan J, Jabbari A, Nguyen N, *et al*. Oral ruxolitinib induces hair regrowth in patients with moderate-to-severe alopecia areata. *JCI Insight* 2016;1:e89790.
46 Barbie TU, Alexe G, Aref AR, *et al*. Targeting an IKBKE cytokine network impairs triple-negative breast cancer growth. *J Clin Invest* 2014;124:5411–23.
47 MedsFacts. Study of possible correlation between systemic lupus erythematosus and genotropin. 2012 http://factmedcom/report-GENOTROPIN-causing-SYSTEMIC%20LUPUS%20ERYTHEMATOSUSphp.
48 Claussnitzer M, Dankel SN, Kim KH, *et al*. FTO Obesity Variant Circuitry and Adipocyte Browning in Humans. *N Engl J Med* 2015;373:895–907.

A disconnect between disease activity and functional ability already in patients with early rheumatoid arthritis, depending on large joint involvement

Current strategies in rheumatoid arthritis (RA) management aim at diagnosing patients and starting disease-modifying antirheumatic drug (DMARD) treatment very early to optimise outcome. International guidelines recommend the initial use of conventional synthetic disease-modifying drugs, especially methotrexate (MTX),[1] even though it has recently been demonstrated that early addition of biologics to the therapeutic regimen with MTX can optimise response.[2] The prevention of functional deterioration is an important treatment goal from both patient and societal perspectives. Previous studies in patients with early RA,[3] and in patients with established RA,[4] demonstrated that large joint involvement is strongly associated with functional impairment and with mortality in long-term observational cohorts.[5]

This post hoc analysis of the U-Act-Early trial was conducted to examine in a cohort of patients with very early RA the impact of large joint involvement (LJI) on disease activity and functional capacity over time. In this trial, 317 patients from 21 Dutch centres were randomised to start treatment with either MTX or tocilizumab (TCZ) monotherapy or with MTX+TCZ combination. The treatment protocol followed a treat-to-target (T2T) strategy with escalation of therapy in patients not achieving sustained remission (defined as a disease activity score assessing 28 joints (DAS28) <2.6 and a swollen joint count of ≤4 joints of the 28 joints assessed, during at least 24 weeks), and de-escalation in those who achieved it. More than 80% of patients who initiated TCZ alone or in combination with MTX achieved sustained remission on this initial regimen versus 44% on MTX monotherapy.[2]

Baseline characteristics of patients with and without LJI (any shoulder, elbow, knee or ankle joint involvement at either screening or baseline visit) are shown in table 1. Patients with LJI had a numerically higher DAS28, significantly higher DAS28-components, assessor's global VAS and number of small joints involved and a higher (worse) health assessment questionnaire (HAQ) score. Other baseline parameters, such as age, gender, disease duration and treatment allocation did not differ between the groups.

The proportion of patients who switched to the next treatment regimen because of insufficient effect of the initial regimen was significantly higher (31% vs 20%, p=0.027) among patients with LJI, but this difference was no longer statistically significant after correction for baseline DAS28. Using longitudinal regression analysis with HAQ over time as outcome, it was estimated that the average score was 0.27 (95% CI 0.39 to 0.15,

Table 1 Baseline characteristics

	No large joint involvement* n=125 patients	Large joint involvement* n=192 patients	p Value
Age (years), mean (SD)	52.6 (12.7)	53.9 (12.9)	0.36
Female gender, n (%)	83 (66.4)	129 (67.2)	0.88
Seropositivity (RF+ or aCCP+), n (%)	99 (79.2)	150 (78.5)	0.89
Disease duration (days), median (Q1, Q3)	27.0 (16.0, 44.0)	25.0 (17.0, 42.0)	0.49
Treatment strategy, n (%)			0.61
MTX+TCZ	42 (33.6)	64 (33.3)	
TCZ	37 (29.6)	66 (34.4)	
MTX	46 (36.8)	62 (32.3)	
ESR (mm/hour), median (Q1, Q3)	18 (11, 28)	33.5 (18, 53.5)	<0.0001
Patient's global VAS (cm), mean (SD), range 0–10=worst	5.2 (2.0)	6.1 (1.9)	0.0002
Assessor's Global VAS (cm), mean (SD), range 0–10=worst†	4.9 (1.8)	6.1 (1.9)	<0.0001
DAS28, mean (SD)	4.7 (0.9)	5.6 (1.1)	0.09
HAQ, mean (SD), range 0–3=worst	0.9 (0.5)	1.3 (0.7)	<0.0001
Tender joint count (28), median (Q1, Q3)	5 (3, 8)	7 (4, 13.5)	0.0002
Swollen joint count (28), median (Q1, Q3)	5 (3, 8)	8 (5, 13)	<0.0001
Type of joints with a swollen joint count >Q1, n (%)			
None	35 (28)	–	
Only large	–	32 (16.7)	
Only small	90 (72)	–	
Both	–	160 (83.3)	
No of large joints involved, median (Q1, Q3)	0	2 (1, 3)	
No of small joints involved, median (Q1, Q3)	9 (6, 13)	14 (9, 18)	<0.0001
No of large joints involved, n patients (% of involvement group)			
0	125 (100)	–	
1–2	–	120 (62.5)	
>2	–	72 (37.5)	

Q1= 25th percentile; Q3=75th percentile.
*Large joint involvement was defined as any shoulder, elbow, knee, ankle joint involvement at either the screening or baseline visit; a 44-joint count was used in the U-Act-Early study.
†Only available for n=95/154.
aCCP, anti-cyclic citrullinated peptide antibody; ESR, erythrocyte sedimentation rate; DAS28, disease activity score assessing 28 joints; HAQ, health assessment questionnaire score; MTX, methotrexate; RF, rheumatoid factor; TCZ, tocilizumab; VAS, visual analogue scale.

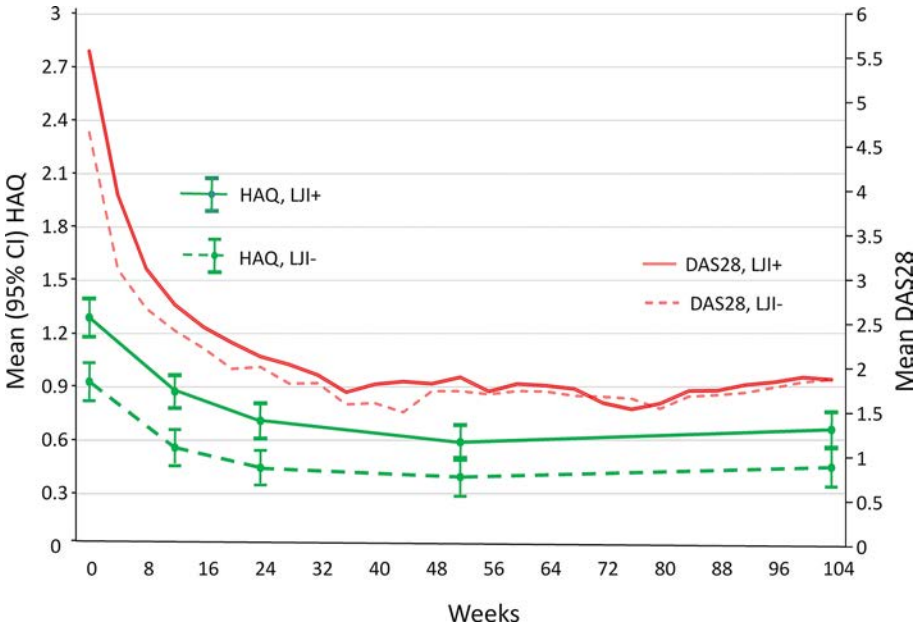

Figure 1 Course of HAQ and DAS28 over time in patients with and patients without large joint involvement (LJI).

p<0.0001) units higher in patients with LJI. The difference remained statistically significant after correction for baseline DAS28 using multivariable regression analysis: 0.17 (95% CI 0.29 to 0.04, p=0.001). Radiographic damage at baseline and after 104 weeks was minimal as described,[2] and deemed not to have influenced HAQ.

Finally, patients without LJI had a higher chance of achieving drug-free remission, independently from baseline DAS28 (odds ratio 1.86, 95% CI 1.04 to 3.3, p=0.036). No statistically significant modification of these associations by treatment strategy was found.

In summary, a significant proportion of patients with very early arthritis had LJI, and this was associated with a higher disease activity at baseline, but not after 2 years following the T2T strategy. In contrast, LJI at baseline resulted in significantly more functional impairment over 2 years, which could not be explained by differences in DAS28 (figure 1). LJI also resulted in a reduced likelihood to achieve drug-free remission. Our findings demonstrate a differential impact of large joints on DAS and HAQ: equal weighting of joints in the DAS28 is contrasting an unequal impact on physical function. This disconnect between disease activity and functional ability depending on the involvement of large joints in this cohort of patients with early RA treated with a T2T strategy demonstrates that disease activity as sole outcome measure cannot capture the whole spectrum of the disease.

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REFERENCES

1 Smolen JS, Landewé R, Bijlsma J, *et al*. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2016 update. *Ann Rheum Dis* 2017;76:960–77.
2 Bijlsma JW, Welsing PM, Woodworth TG, *et al*. Early rheumatoid arthritis treated with tocilizumab, methotrexate, or their combination (U-Act-Early): a multicentre, randomised, double-blind, double-dummy, strategy trial. *Lancet* 2016;388:343–55.
3 Kuper HH, van Leeuwen MA, van Riel PL, *et al*. Radiographic damage in large joints in early rheumatoid arthritis: relationship with radiographic damage in hands and feet, disease activity, and physical disability. *Br J Rheumatol* 1997;36:855–60.
4 Drossaers-Bakker KW, Kroon HM, Zwinderman AH, *et al*. Radiographic damage of large joints in long-term rheumatoid arthritis and its relation to function. *Rheumatology* 2000;39:998–1003.
5 Pincus T, Brooks RH, Callahan LF. Prediction of long-term mortality in patients with rheumatoid arthritis according to simple questionnaire and joint count measures. *Ann Intern Med* 1994;120:26–34.

The extensive glycosylation of the ACPA variable domain observed for ACPA-IgG is absent from ACPA-IgM

Recently, we described the presence of highly sialylated *N*-linked glycans in the antigen-binding fragment (Fab) of almost all anti-citrullinated protein antibody (ACPA) IgG molecules.[1 2] These glycans could not be found on several other autoantibody systems analysed. Given the low affinity of ACPA,[3] this observation raises the intriguing possibility that citrullinated antigen-specific B cells could be selected based on the presence of glycans in the variable domain, rather than on affinity for their cognate antigen. *N*-glycosylation requires the presence of specific consensus sequences in the amino acid backbone of proteins.[4] However, only few human germline Ig variable region genes encode for such sequences.[5] So far, we could identify several *N*-glycosylation sites in ACPA-IgG Fab-domains using mass spectrometry, but none of these were encoded in the germline sequence.[1] This suggests that the extensive presence of *N*-glycans in ACPA-IgG Fab-domains results from somatic mutations. Moreover, it indicates that the ACPA response matures under the influence of T-cell help, presumably in germinal centres, and makes it conceivable that the introduction of *N*-glycosylation sites might be a crucial step by which tolerance checkpoints are breached. In this respect, it is relevant to note that ACPA are frequently present before the onset of clinical symptoms, and that recent epidemiological data indicate that the Human Leukocyte Antigen (HLA) region is not a genetic risk factor for the development of ACPA, but associates strongly with ACPA-positive rheumatoid arthritis (RA).[6 7] Thus, the HLA region and thereby T-cells contribute primarily to the maturation of the ACPA response rather than to its presence. If correct, additional glycosylation of the variable domain should be absent from ACPA-IgM. Here, we tested this hypothesis to gain insight in the biological processes underlying the extensive Fab-glycosylation of ACPA-IgG. ACPA-positive sera from patients with established RA were fractionated by size using gel-filtration chromatography and tested for the presence of ACPA isotypes by ELISA.[1] Furthermore, ACPA were affinity purified from synovial fluid and plasma followed by the heavy chain detection of ACPA isotypes by western blot.[8] Finally, the presence or absence of sialylated Fab-glycans on ACPA was tested by Sambucus nigra lectin (SNA) chromatography.[9] ACPA-IgG and ACPA-IgG1 consistently showed an increased molecular weight compared with non-citrulline-specific IgG, consistent with the presence of Fab-glycans (figure 1A,B (1)). In

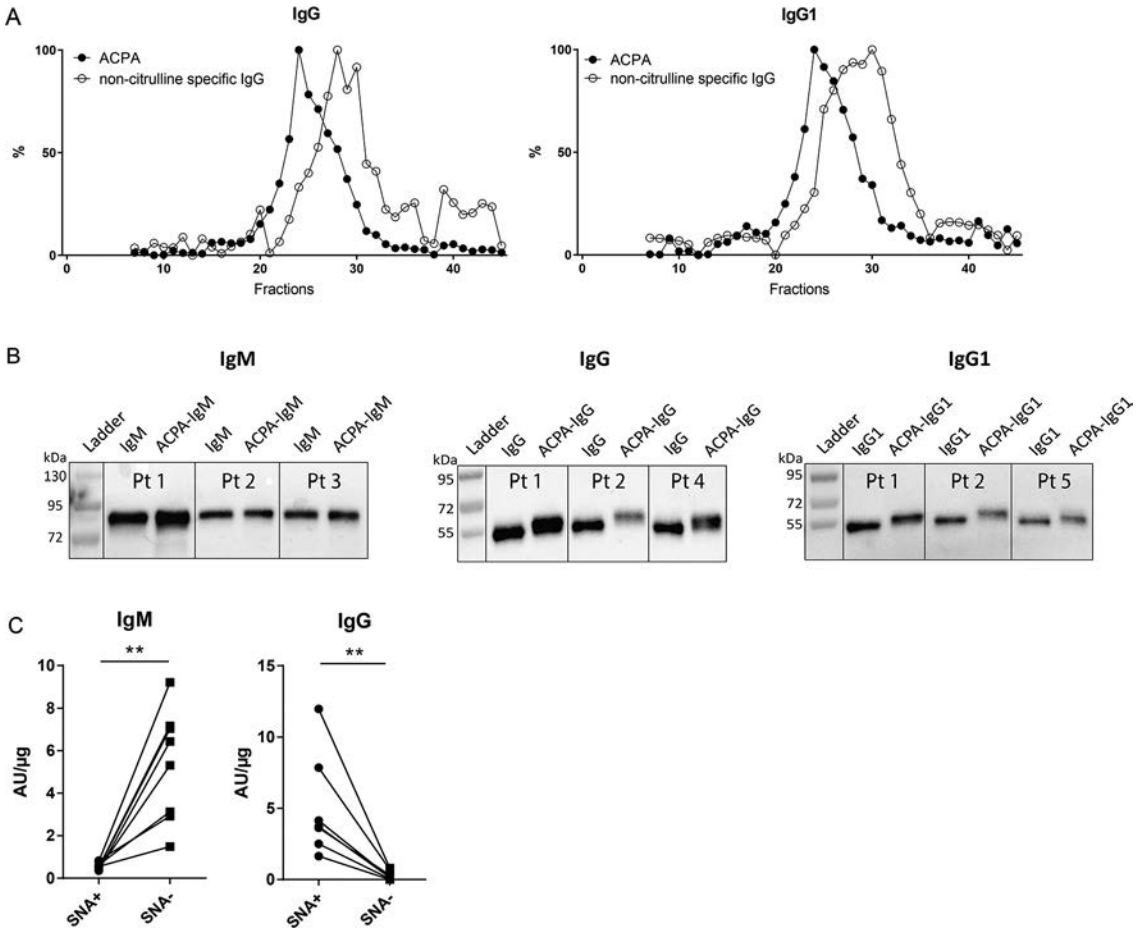

Figure 1 Molecular size analysis of ACPA-IgG and -IgM. (A) Presence of anti-citrullinated protein antibody (ACPA)-IgG (black dots) and non-citrulline-specific IgG (white dots) in gel-filtration chromatography fractions (lower fraction number indicates larger size; representative example of seven donors). (B) Western blot of ACPA and non-citrulline-specific control Ig isolated from patients (Pt) with established rheumatoid arthritis (RA) and stained for IgM, IgG and IgG1 (n=7). (C) Ratio of ACPA-IgM and ACPA-IgG (in AU) per non-citrulline-specific IgM and IgG (in µg) in Sambucus nigra lectin (SNA) positive (SNA+) and SNA-negative (SNA-) fractions (obtained using sera from n=8 additional donors; non-parametric Wilcoxon test for matched pairs, **p<0.01).

contrast, ACPA-IgM were identical in size compared with their non-citrulline-specific counterparts (figure 1B). ACPA-IgG Fab-glycans are highly sialylated[2] and therefore likely to interact with SNA. Consequently, ACPA-IgG could be strongly enriched on SNA purification, while ACPA-IgM remained in the SNA-negative fraction (figure 1C) indicating the absence of such glycan species from ACPA-IgM. We conclude that the absence of a molecular 'size-shift' for ACPA-IgM suggests that this ACPA isotype lacks additional glycosylation in the variable region. Unlike ACPA-IgG, ACPA-IgM seems to have an overall lower degree of sialylation independent of Fab glycosylation. These results are compatible with the notion that the acquisition of Fab-glycans in the variable domain of ACPA-IgG reflects a T-cell dependent process in the development of citrullinated antigen-specific B cells in RA. In future studies, it will be crucial to understand the potential selective survival signals these glycans confer on ACPA-expressing B cells.

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REFERENCES

1 Rombouts Y, Willemze A, van Beers JJ, *et al*. Extensive glycosylation of ACPA-IgG variable domains modulates binding to citrullinated antigens in rheumatoid arthritis. *Ann Rheum Dis* 2016;75:578–85.
2 Hafkenscheid L, Bondt A, Scherer HU, *et al*. Structural analysis of variable domain Glycosylation of Anti-Citrullinated protein antibodies in rheumatoid Arthritis Reveals the Presence of Highly Sialylated Glycans. *Mol Cell Proteomics* 2017;16:278–87.
3 Suwannalai P, Scherer HU, van der Woude D, *et al*. Anti-citrullinated protein antibodies have a low avidity compared with antibodies against recall antigens. *Ann Rheum Dis* 2011;70:373–9.
4 Kasturi L, Eshleman JR, Wunner WH, *et al*. The hydroxy amino acid *in an* As*n*-*X*-Ser/Thr sequ*on can* influence *N*-linked core glycosylati*on* efficiency and the level of expressi*on* of a cell surface glycoprotein. *J Biol Chem* 1995;270:14756–61.
5 van de Bovenkamp FS, Hafkenscheid L, Rispens T, *et al*. The emerging Importance of IgG Fab Glycosylation in immunity. *J Immunol* 2016;196:1435–41.
6 Hensvold AH, Magnusson PK, Joshua V, *et al*. Environmental and genetic factors in the development of anticitrullinated protein antibodies (ACPAs) and ACPA-positive rheumatoid arthritis: an epidemiological investigation in twins. *Ann Rheum Dis* 2015;74:375–80.
7 Terao C, Ohmura K, Ikari K, *et al*. Effects of smoking and shared epitope on the production of anti-citrullinated peptide antibody in a Japanese adult population. *Arthritis Care Res* 2014;66:1818–27.
8 Willemze A, Shi J, Mulder M, *et al*. The concentration of anticitrullinated protein antibodies in serum and synovial fluid in relation to total immunoglobulin concentrations. *Ann Rheum Dis* 2013;72:1059–63.
9 Käsermann F, Boerema DJ, Rüegsegger M, *et al*. Analysis and functional consequences of increased Fab-sialylation of intravenous immunoglobulin (IVIG) after lectin fractionation. *PLoS One* 2012;7:e37243.:e37243.

Inflammatory features of infrapatellar fat pad in rheumatoid arthritis versus osteoarthritis reveal mostly qualitative differences

Rheumatoid arthritis (RA) and osteoarthritis (OA) are characterised by joint destruction. In both diseases, inflammation is implicated; however, RA is generally associated with more inflammation in synovium[1] and synovial fluid (SF)[2–4] compared with OA. The infrapatellar fat pad (IFP), an adipose tissue located in the knee joint, has been proposed to contribute to disease progression in OA. Although IFP of OA has been extensively characterised and identified as a source of inflammation in the joint,[5–7] only scarce information is available on IFP in patients with RA. Therefore, our aim was to get a first insight into the possible contribution of IFP to the inflammatory processes in the RA joint.

To this end, we compared leftover IFP and synovium from patients with RA (n=20: 80% women, mean (SD) age 64 (10.7) years, median (range) body mass index (BMI) 26.9 (18) kg/m^2) and patients with primary knee OA (n=51: 62% women, mean (SD) age 66 (8.9) years, median (range) BMI 28.9 (15) kg/m^2); all were undergoing knee joint replacement surgery. The study was approved by the local medical ethical committee. Fat-conditioned medium (FCM) and adipocyte-conditioned medium (ACM) were generated as previously described.[5] Adipokines and cytokines were measured in FCM and ACM with Milliplex MAP Human Adipocyte kit (Millipore) and Milliplex MAP Human Adipokine kit (Millipore), using the Bio-Plex array reader and Bio-Plex software according to the manufacturer's instructions. Stromal vascular fraction (SVF) was isolated and cells were identified by flow cytometry as previously described.[5] The number of mast cells in IFP was determined based on fluorescence-activated cell sorting (FACS) and the number of SVF cells counted on digestion. Synovitis score and number of synovial CD117$^+$ cells

Table 1 Levels of adipocytokines in FCM and ACM. Depicted are median (Q1–Q3). Difference between RA and OA group determined with Mann-Whitney are indicated with p value.

			RA		OA		p Value
			Median	(Q1–Q3)	Median	(Q1–Q3)	
FCM			n=11		n=22		
	Adipsin	(ng/ml)	382.9	(226.3–457.9)	349.9	(215.2–446.3)	0.667
	Adiponectin	(ng/ml)	103.1	(87.5–124.8)	97.7	(64–193.8)	0.585
	Leptin	(ng/ml)	3.3	(0.7–4.1)	1.4	(0.7–2.4)	0.233
	Resistin	(pg/ml)	66.8	(36.5–248.6)	90.6	(57.4–186.6)	0.618
	IL6	(ng/ml)	29.7	(8.5–63.5)	10.1	(1.9–19.8)	0.076
	IL8	(ng/ml)	18.5	(3.4–74.5)	8.3	(1.6–20.7)	0.133
	MCP-1	(ng/ml)	11.6	(9.9–12.2)	9.2	(5.5–13.1)	0.349
	TNFα	(pg/ml)	2.0	(0.8–12.6)	1.8	(0.9–8.3)	0.873
	IL6R	(pg/ml)	13.6	(5.7–22.3)	20.9	(11.2–31.4)	0.182
ACM			n=13		n=21		
	Adipsin	(ng/ml)	22.6	(10.8–34.1)	30.3	(20.5–44.2)	0.129
	Adiponectin	(ng/ml)	21.8	(11.8–41.0)	34.5	(22.6–51.3)	0.076
	Leptin	(pg/ml)	255.8	(98.9–797.9)	318.5	(149.4–658.5)	0.821
	Resistin	(pg/ml)	2.7	(2.7–7.0)	2.7	(1.2–4.3)	0.255
	IL6	(ng/ml)	0.4	(0.2–1.5)	1.1	(0.3–2.2)	0.344
	IL8	(ng/ml)	0.6	(0.4–2.0)	2.1	(0.6–3.2)	0.193
	MCP-1	(ng/ml)	0.3	(0.1–0.6)	0.7	(0.3–1)	0.089
	TNFα	(pg/ml)	3.4	(1.4–9.8)	8.3	(3.5–13.5)	0.156
	IL6R	(pg/ml)	ND		ND		

ACM, adipocyte-conditioned medium; FCM, fat-conditioned medium, IL, interleukin; MCP, monocyte chemoattractant protein-1; ND, not detectable; OA, osteoarthritis; RA, rheumatoid arthritis; TNFα, tumour necrosis factor alpha.

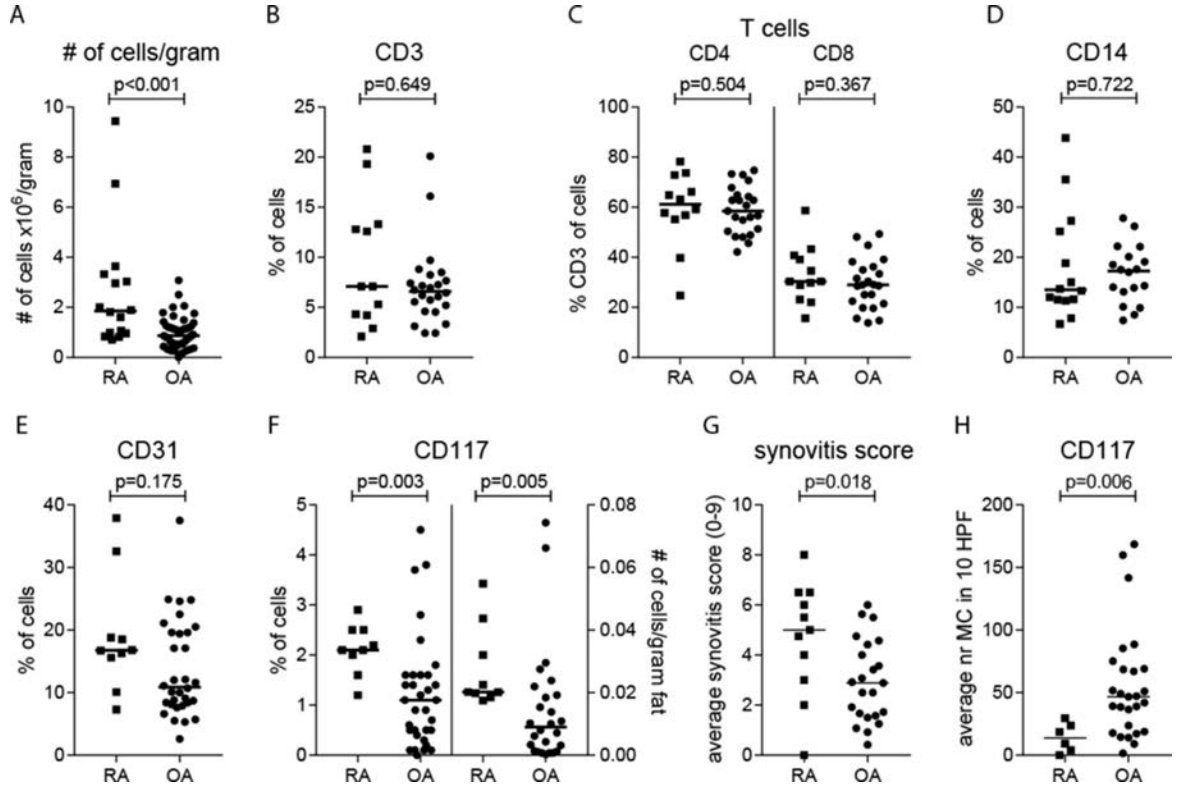

Figure 1 Immune cell quantification and characterisation of the stromal vascular fraction (SVF) of the infrapatellar fat pad (IFP) of patients with rheumatoid arthritis (RA) and osteoarthritis (OA). SVF was isolated and number of cells per gram fat tissue was determined (A). Using flow cytometry, the percentage of $CD3^+$ (B), $CD4^+$ and $CD8^+$ (C), $CD14^+$ (D), $CD31^+$ (E) and $CD117^+$ (F) cells were determined. The number of $CD117^+$ was calculated using the percentage of $CD117^+$ cells and the number of cells per gram of tissue (F). Synovitis score (G) and the number of $CD117^+$ cells (H) was determined using immunohistochemistry or immunofluorescence, respectively. Median is represented and each dot represents one patient. According to Bonferroni correction for multiple testing, a p value of <0.01, determined by Mann-Whitney was considered statistically significant for (A)–(F), while a p value of 0.025, determined by Mann-Whitney was considered statistically significant for (G)–(H). HPF, high power field; MC, mast cell.

was determined as previously described.[1] OA and RA groups were matched for age, gender and BMI on group level for FCM, ACM, FACS analysis, immunohistochemistry and immunofluorescence analysis.

The IFP (FCM) and adipocytes (ACM) of patients with OA and RA secreted various adipocytokines; however, no significant differences were observed (table 1). The IFP of patients with RA contained a higher number of cells than IFP of patients with OA (figure 1A), but the percentages of T cells ($CD3^+$, figure 1B), $CD4^+$ or $CD8^+$ T cells (figure 1C), monocytes ($CD14^+$, figure 1D) and endothelial cells ($CD31^+$, figure 1E) were comparable between patients with RA and OA. The percentage of B cells was below 1% in both populations (data not shown). The only significant difference was observed in the percentage and abundance ($CD117^+$, figure 1F) of mast cells, which were higher in RA IFP. Interestingly, although RA synovium had a higher synovitis score (figure 1G), lower numbers of mast cells were found in RA synovium compared with OA synovium (figure 1H and de Lange-Brokaar *et al*[1]), suggesting that mast cell numbers are controlled by different signals in OA and RA.

Despite the small sample size, our data indicate that the higher cellular infiltrate in RA compared with OA IFP does not result in clear differences in secretory profile of these tissues, indicating a limited contribution of infiltrating immune cells to secretion of the investigated adipocytokines in these patients. However, our data await further replication and should not be extrapolated to earlier stages of RA as inflammation in all joint tissues could be different at an earlier disease stage. Moreover, as patients with RA usually display a higher SF and synovial inflammatory cytokine load than patients with OA,[4 8] this is not readily apparent for IFP in this study. Although the lack of SF is a limitation of our study, these data suggest little contribution of IFP to SF cytokines in RA. The increased cellular infiltration of RA IFP could be a reflection of the generally higher inflammatory load present in the joint of patients with RA.

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Correction notice This article has been corrected since it published Online First. The median level of IL6 in FCM of OA patients in table 1 has been corrected to 10.1ng/ml.

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REFERENCES

1 de Lange-Brokaar BJ, Kloppenburg M, Andersen SN, *et al*. Characterization of synovial mast cells in knee osteoarthritis: association with clinical parameters. *Osteoarthritis Cartilage* 2016;24:664–71.
2 Krenn V, Morawietz L, Burmester GR, *et al*. Synovitis score: discrimination between chronic low-grade and high-grade synovitis. *Histopathology* 2006;49:358–64.
3 Ropes MW, Bauer W. *Synovial fluid changes in joint disease*: Harvard University Press, 1953.
4 Jónasdóttir HS, Brouwers H, Kwekkeboom JC, *et al*. Targeted lipidomics reveals activation of resolution pathways in knee osteoarthritis in humans. *Osteoarthritis Cartilage* 2017;25:1150–60.
5 Klein-Wieringa IR, Kloppenburg M, Bastiaansen-Jenniskens YM, *et al*. The infrapatellar fat pad of patients with osteoarthritis has an inflammatory phenotype. *Ann Rheum Dis* 2011;70:851–7.
6 Distel E, Cadoudal T, Durant S, *et al*. The infrapatellar fat pad in knee osteoarthritis: an important source of interleukin-6 and its soluble receptor. *Arthritis Rheum* 2009;60:3374–7.
7 Eymard F, Pigenet A, Citadelle D, *et al*. Induction of an inflammatory and prodegradative phenotype in autologous fibroblast-like synoviocytes by the infrapatellar fat pad from patients with knee osteoarthritis. *Arthritis Rheumatol* 2014;66:2165–74.
8 de Lange-Brokaar BJ, Ioan-Facsinay A, van Osch GJ, *et al*. Synovial inflammation, immune cells and their cytokines in osteoarthritis: a review. *Osteoarthritis Cartilage* 2012;20:1484–99.

Evaluation of the effect of baseline MRI sacroiliitis and C reactive protein status on etanercept treatment response in non-radiographic axial spondyloarthritis: a post hoc analysis of the EMBARK study

The efficacy of tumour necrosis factor alpha (TNFα) inhibitors for patients with non-radiographic axial spondyloarthritis (nr-axSpA) has now been firmly established[1–3]; however, identification of objective markers, predictive of treatment response, will be of considerable benefit in optimising patient outcomes and ensuring the most appropriate treatment prioritisation and resource allocation. We have investigated this further using the findings of EMBARK (effect of etanercept on symptoms and objective inflammation in nr-axSpA, a 104 week study) (ClinicalTrials.gov identifier: NCT01258738), a phase IIIb, 104-week randomised controlled trial in >200 patients with nr-axSpA,[4–6] which showed a possible association between higher baseline C reactive protein (CRP) levels or magnetic resonance imaging (MRI) sacroiliac joint (SIJ) scores and a placebo (PBO)-adjusted treatment effect. The objective of this post hoc analysis was to determine whether MRI sacroiliitis (positive/negative (+/−)) and/or high-sensitivity CRP (hs-CRP) (elevated/normal (+/−)) at baseline are predictive of changes in measures of disease activity on etanercept (ETN) treatment in patients with nr-axSpA.

Eligible patients were randomised to 12 weeks' double-blind treatment with 50 mg ETN once weekly or PBO. Both groups continued stable non-steroidal anti-inflammatory drug (NSAID) therapy. MRI scanning of the SIJ and spine was performed at screening and at week 12. The primary efficacy endpoint was the percentage of Assessment of SpondyloArthritis International Society (ASAS) 40% (ASAS40) responders at week 12.[7]

A total of 215 patients (ETN, n=106; PBO, n=109)[4] were included (MRI−/CRP− (n=26); MRI+/CRP− (n=97); MRI−/CRP+ (n=15); MRI+/CRP+ (n=77)). Week 12 outcomes were achieved by a higher proportion of patients receiving ETN than PBO. The greatest ETN response was seen in the MRI+/CRP+ subgroup and possibly MRI−/CRP+ (although the sample size is small). PBO response was greatest in the MRI+/CRP+ subgroup; lowest response for both treatment arms was seen in the MRI−/CRP− subgroup (figure 1), consistent with regression to the mean effects.

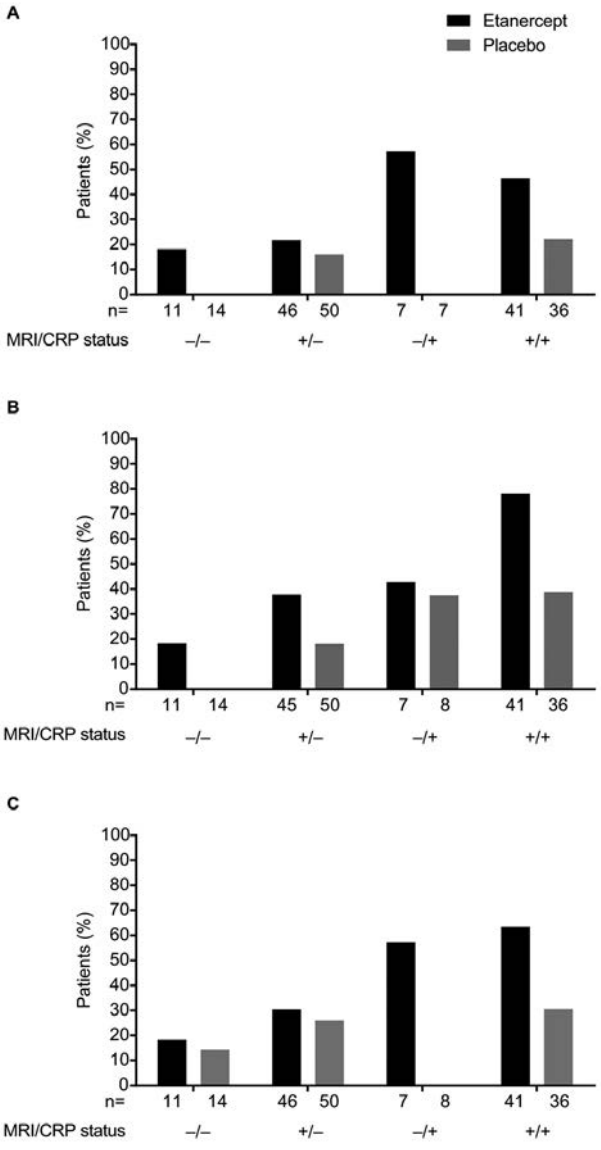

Figure 1 Responses from baseline to week 12. (A) proportion of patients who achieved an ASAS40 response; (B) proportion of patients who achieved an ASDAS-CRP (Δ ≥1.1) response; (C) proportion of patients who achieved a BASDAI50 response. ASAS, Assessment of SpondyloArthritis International Society; ASDAS, Ankylosing Spondylitis Disease Activity Score; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; CRP, C reactive protein; MRI, magnetic resonance imaging.

Based on treatment-by-predictor interaction p values, using logistic regression models (table 1), CRP high versus low, continuous CRP, unilateral grade =II versus <II, elevated CRP and ASAS MRI sacroiliitis (yes vs no), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) ≥median versus <median of 6.17, HLA-B27 positive versus negative and Europe versus other regions had a significant interaction with treatment ($p<0.10$) for one or both endpoints (ASAS40, Ankylosing Spondylitis Disease Activity Score-CRP <2.1). The best set of baseline predictors of week 12 ASAS40 response in the stepwise logistic regression model was ETN treatment (OR (95% CI), 3.02 (1.45 to 6.27); $p<0.01$), elevated CRP (>3 mg/L) (2.50 (1.20 to 5.20); $p<0.05$) and low Spondyloarthritis Research Consortium of Canada (SPARCC) SIJ score (0.96 (0.93 to 0.10); $p<0.05$).

Although evidence suggests responses to TNFα inhibitors[1 2] are greater in patients with elevated hs-CRP and MRI-documented SIJ inflammation, this analysis is the first to directly assess response to ETN treatment in patients with nr-axSpA, based on MRI sacroiliitis and CRP status at baseline. Based on significant ORs, our analyses suggest that in patients with an inadequate response to ≥2 NSAIDs, elevated hs-CRP at baseline, alone or combined with MRI positivity, alongside being male, unilateral grade II, higher SPARCC SIJ score and lower BASDAI score, were associated with greater improvement in joint inflammation scores and clinical responses of ETN-treated patients. Significant interaction p values and ORs were not adjusted for multiple comparisons and so should be interpreted with caution. Further study in a larger patient sample is warranted to definitively establish these findings.

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Table 1 Baseline predictors of 12-week ASAS40 and 12-week ASDAS-CRP <2.1 (mITT population)*

Baseline predictor (subgroup 2 vs 1)	Treatment	Patients, n/N (%) ASAS40 Subgroup 2	ASAS40 Subgroup 1	ASAS40 OR (95% CI)†	ASAS40 p Value‡	ASDAS-CRP<2.1 Subgroup 2	ASDAS-CRP<2.1 Subgroup 1	ASDAS-CRP<2.1 OR (95% CI)†	ASDAS-CRP<2.1 p Value‡
Age (>40 y vs ≤40 y)	ETN	3/15 (20.0)	32/90 (35.6)[b]	0.45 (0.12 to 1.73)	>0.10	9/15 (60.0)	60/90 (66.7)[b]	0.75 (0.24 to 2.30)	>0.10
	PBO	1/17 (5.9)	15/90 (16.7)	0.31 (0.04 to 2.54)		7/17 (41.2)	42/92 (45.7)	0.83 (0.29 to 2.38)	
Sex (male vs female)	ETN	24/68 (35.3)[a]	11/37 (29.7)[a]	1.29 (0.54 to 3.06)	>0.10	52/68 (76.5)[b]	17/37 (45.9)	3.82 (1.63 to 9.00)[a]	0.08
	PBO	12/62 (19.4)	4/45 (8.9)	2.46 (0.74 to 8.21)		30/62 (48.4)	19/47 (40.4)	1.38 (0.64 to 2.97)	
MRI sacroiliitis by ASAS criteria (positive vs negative)	ETN	29/87 (33.3)	6/18 (33.3)	1.00 (0.34 to 2.94)	>0.10	58/87 (66.7)[b]	11/18 (61.1)	1.27 (0.45 to 3.63)	>0.10
	PBO	16/86 (18.6)	0/21 (0.0)	NA		40/87 (46.0)	9/22 (40.9)	1.23 (0.48 to 3.18)	
Elevated CRP (>3 mg/L) (high vs low)	ETN	23/48 (47.9)[b]	12/57 (21.1)	3.45 (1.47 to 8.09)[a]	>0.10	32/48 (66.7)[c]	37/57 (64.9)	1.08 (0.48 to 2.43)	0.02
	PBO	8/43 (18.6)	8/63 (12.7)	1.57 (0.54 to 4.57)		12/44 (27.3)	37/64 (57.8)	0.27 (0.12 to 0.63)[a]	
CRP continuous	ETN			2.09 (1.27 to 3.45)[a]	0.03			0.96 (0.66 to 1.40)	0.03
	PBO			0.93 (0.53 to 1.62)				0.43 (0.22 to 0.81)[a]	
Unilateral grade (=II vs <II)	ETN	20/41 (48.8)[b]	15/64 (23.4)	3.11 (1.34 to 7.22)[a]	0.04	29/41 (70.7)	40/64 (62.5)[a]	1.45 (0.63 to 3.37)	>0.10
	PBO	4/35 (11.4)	12/72 (16.7)	0.65 (0.19 to 2.17)		17/35 (48.6)	32/74 (43.2)	1.24 (0.55 to 2.78)	
Elevated CRP AND ASAS MRI sacroiliitis (yes vs no)	ETN	19/41 (46.3)[a]	16/64 (25.0)[a]	2.59 (1.12 to 5.97)[a]	>0.10	28/41 (68.3)[c]	41/64 (64.1)	1.21 (0.53 to 2.78)	0.03
	PBO	8/36 (22.2)	8/70 (11.4)	2.21 (0.75 to 6.50)		10/36 (27.8)	39/72 (54.2)	0.33 (0.14 to 0.77)[a]	
BASDAI total score continuous	ETN			1.20 (0.94 to 1.54)	>0.10			0.65 (0.49 to 0.86)[a]	>0.10
	PBO			1.07 (0.80 to 1.43)				0.49 (0.36 to 0.66)[a]	
BASDAI ≥median vs <median of 6.17	ETN	20/53 (37.7)[b]	15/52 (28.8)	1.50 (0.66 to 3.39)	>0.10	29/53 (54.7)[c]	40/52 (76.9)	0.36 (0.16 to 0.84)[a]	0.08
	PBO	7/51 (13.7)	9/56 (16.1)	0.83 (0.29 to 2.42)		11/53 (20.8)	38/56 (67.9)	0.12 (0.05 to 0.30)[a]	
HLA-B27 (positive vs negative)	ETN	27/70 (38.6)[c]	7/33 (21.2)	2.33 (0.89 to 6.11)	>0.10	50/70 (71.4)[c]	17/33 (51.5)	2.35 (1.00 to 5.55)	0.05
	PBO	11/81 (13.6)	3/23 (13.0)	1.05 (0.27 to 4.12)		35/83 (42.2)	12/23 (52.2)	0.67 (0.27 to 1.69)	
SPARCC SIJ (≥2 vs<2)	ETN	28/67 (41.8)[b]	5/28 (17.9)	3.30 (1.12 to 9.75)[a]	>0.10	47/67 (70.1)[c]	17/28 (60.7)	1.52 (0.61 to 3.82)	>0.10
	PBO	13/73 (17.8)	3/30 (10.0)	1.95 (0.51 to 7.41)		31/74 (41.9)	15/31 (48.4)	0.77 (0.33 to 1.79)	
SPARCC continuous	ETN			1.08 (1.03 to 1.14)[a]	>0.10			1.09 (1.01 to 1.16)[a]	0.10
	PBO			1.03 (0.98 to 1.08)				1.02 (0.98 to 1.06)	
Concomitant DMARD use (yes vs no)	ETN	6/21 (28.6)	29/84 (34.5)[b]	0.76 (0.27 to 2.16)	>0.10	16/21 (76.2)[a]	53/84 (63.1)[a]	1.87 (0.63 to 5.61)	>0.10
	PBO	2/20 (10.0)	14/87 (16.1)	0.58 (0.12 to 2.78)		8/21 (38.1)	41/88 (46.6)	0.71 (0.27 to 1.87)	
Europe vs other regions	ETN	29/78 (37.2)[c]	6/27 (22.2)	2.07 (0.75 to 5.73)	0.06	50/78 (64.1)[b]	19/27 (70.4)	0.75 (0.29 to 1.94)	>0.10
	PBO	9/75 (12.0)	7/32 (21.9)	0.49 (0.16 to 1.45)		30/77 (39.0)	19/32 (59.4)	0.44 (0.19 to 1.01)	

*For each treatment arm, both continuous and dichotomous baseline predictors were analysed in univariate logistic models of either week 12 ASAS40 or ASDAS-CRP<2.1 response.
†OR for subgroup 2 versus subgroup 1 within each treatment arm ([a]p<0.05).
‡p Value for treatment by subgroup interaction ([a]p<0.05, [b]p<0.01, or [c]p<0.001 between treatments within subgroup).
ASAS, Assessment of SpondyloArthritis International Society; ASDAS, Ankylosing Spondylitis Disease Activity Score; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; CRP, C reactive protein; DMARD, disease-modifying antirheumatic drug; ETN, etanercept; HLA, human leucocyte antigen; mITT, modified intent-to-treat; MRI, magnetic resonance imaging; NA, not applicable; PBO, placebo; SIJ, sacroiliac joint; SPARCC, Spondyloarthritis Research Consortium of Canada; y, years.

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REFERENCES

1 Sieper J, van der Heijde D, Dougados M, *et al*. Efficacy and safety of adalimumab in patients with non-radiographic axial spondyloarthritis: results of a randomised placebo-controlled trial (ABILITY-1). *Ann Rheum Dis* 2013;72:815–22.
2 Sieper J, van der Heijde D, Dougados M, *et al*. A randomized, double-blind, placebo-controlled, sixteen-week study of subcutaneous golimumab in patients with active nonradiographic axial spondyloarthritis. *Arthritis Rheumatol* 2015;67:2702–12.
3 Landewé R, Braun J, Deodhar A, *et al*. Efficacy of certolizumab pegol on signs and symptoms of axial spondyloarthritis including ankylosing spondylitis: 24-week results of a double-blind randomised placebo-controlled phase 3 study. *Ann Rheum Dis* 2014;73:39–47.
4 Dougados M, van der Heijde D, Sieper J, *et al*. Symptomatic efficacy of etanercept and its effects on objective signs of inflammation in early nonradiographic axial spondyloarthritis: a multicenter, randomized, double-blind, placebo-controlled trial. *Arthritis Rheumatol* 2014;66:2091–102.
5 Maksymowych WP, Dougados M, van der Heijde D, *et al*. Clinical and MRI responses to etanercept in early non-radiographic axial spondyloarthritis: 48-week results from the EMBARK study. *Ann Rheum Dis* 2016;75:1328–35.
6 Wei JC, Tsai WC, Citera G, *et al*. Efficacy and safety of etanercept in patients from Latin America, Central Europe and Asia with early non-radiographic axial spondyloarthritis. *Int J Rheum Dis* 2016 [Epub ahead of print].
7 Rudwaleit M, van der Heijde D, Landewé R, *et al*. The development of Assessment of SpondyloArthritis International Society classification criteria for axial spondyloarthritis (part II): validation and final selection. *Ann Rheum Dis* 2009;68:777–83.

Serum neurofilament light chain: a biomarker of neuronal injury in vasculitic neuropathy

Peripheral nerve biopsy remains the gold standard for diagnosing vasculitic neuropathy (VN).[1] This procedure is invasive, of limited sensitivity and not always feasible.[2] A biomarker indicating acute axonal loss in patients with systemic vasculitis would allow rapid screening and adjustments of diagnostic or treatment strategies, potentially improving clinical outcome.[3]

Neurofilaments are structural proteins specific to neurons that are released into blood and cerebrospinal fluid following neuronal damage. They have been suggested as a biomarker in various neurological diseases, mainly affecting the central nervous system.[4–6] In patients with multiple sclerosis, serum neurofilament light chain (sNfL) levels correlated with MRI and clinical disease activity/severity, were lower in patients under treatment and showed potential to predict future disease activity and disability.[5]

In this retrospective study we aimed to test whether sNfL could serve as a marker of vasculitic damage of peripheral nerves and/or disease activity in patients with VN. Patients were recruited from our prospective local ethical board-approved vasculitis cohort. VN was diagnosed by biopsy or, in case biopsy was declined or yielded indefinite results, by clinical presentation.[1] Neurological disability was assessed using the Medical Research Council Sum Score, measuring motor function of 12 key limb muscles (normal exam at maximum score 60), and the Neurological Symptom Score, scoring cranial nerve deficits, motor function, positive and negative sensory and autonomic symptoms (maximum disability at score of 17).[7 8] Systemic vasculitic disease activity was determined by the Birmingham Vasculitis Activity Index.[9] VN remission was defined as stable or improved neurological exam.

sNfL levels were determined by a novel ultrasensitive Simoa[5] assay in a 10-patient cohort at initial presentation with active VN and subsequent remission (table 1). Serial samples were available before onset of neuropathy in one cohort patient (patient 8) and an additional relapsing patient (patient 11).

sNfL levels at diagnosis were compared with 30 age-matched and sex-matched healthy controls (HC), 10 age-matched controls at diagnosis of systemic vasculitis without neuropathy (VC) and 3 patients with non-vasculitic neuropathy (NC) (diabetic, idiopathic and paraneoplastic, respectively).

At the time of sampling, all patients of the VN and VC groups had active disease. sNfL levels were higher in the VN patients at diagnosis (median 215; range 74–2364 pg/mL) compared with HC (median 29; range 10–64 pg/mL, $p<0.001$), NC (median 64; range 34–73 pg/mL, $p\sim0.028$) and VC (median 43; range 15–147 pg/mL, $p<0.001$) (figure 1A). At VN diagnosis sNfL levels were significantly higher than during remission (median 57; range 12–83 pg/mL; $p=0.002$) (figure 1B). In patients 8 and 11 (table 1) sNfL levels clearly paralleled clinical course before VN onset (patient 8) and relapse (patient 11), respectively, and during active disease and remission (figure 1C).

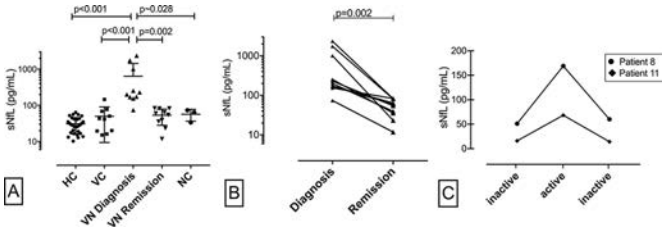

Figure 1 (A) Median sNfL levels in patients with vasculitic neuropathy at diagnosis (VN diagnosis) and during remission (VN remission), healthy controls (HC), patients with vasculitis without neuropathy (VC) and patients with non-vasculitic neuropathy (NC) (whiskers indicate IQR range). (B) Longitudinal measurement of sNfL levels at diagnosis and during remission in patients with vasculitic neuropathy and (C) sNfL levels for VN patient 8 (circle) before, at onset and in remission, and patient 11 (diamond) during remission, at relapse and during subsequent remission. Pairwise Wilcoxon rank-sum test was performed. Holm correction was applied to correct for multiple comparisons. sNfL, serum neurofilament light chain.

Table 1 Characteristics of patients with vasculitic neuropathy at onset and follow-up

Patient ID	Age (years)	Sex	Disease	Histo	Pattern	Therapy	FU (months)	sNfL (pg/mL)		BVAS (0–68)		NSS (0–17)		MRC (0–60)	
								Onset	FU	Onset	FU	Onset	FU	Onset	FU
1	65	F	SS, Cryo	ND	AS	RTX	11	2364	82	29	0	3	2	59	60
2	77	M	MPA	ND	AS	CYC, MMF	15	1754	82	12	0	6	4	55	57
3	43	F	GPA	–	MM	RTX, CYC	18	1014	23	16	0	3	3	57	59
4	62	M	NSVN	++	MM	CYC, RTX	13	254	36	NA	NA	5	4	39	41
5	69	F	SS	+	AS	MTX, RTX	11	230	55	9	0	3	3	60	60
6	60	F	MPA	++	AS	RTX	14	200	40	9	0	4	2	ND	59
7	73	M	Cryo	++	SY	RTX, CYC	10	174	64	8	0	1	1	60	60
8	62	F	GPA	++	MM	CYC	10	169	60	12	0	5	3	55	60
9	64	M	GPA	++	MM	RTX	12	155	83	24	0	4	2	55	59
10	27	F	NSVN	++	MM	AZA	13	74	12	NA	NA	2	2	60	60
11	28	F	NSVN	++	MM	AZA, CYC, MMF	74	68	14	NA	NA	4	2	56	58

AS, asymmetric neuropathy; AZA, azathioprine; BVAS, Birmingham Vasculitis Activity Scale; Cryo, cryoglobulinaemia; CYC, cyclophosphamide; F, female; FU, follow-up during remission; GPA, granulomatosis with polyangiitis; Histo, biopsy findings according to the Peripheral Nerve society Guidelines; M, male; MM, mononeuritis multiplex; MMF, mycophenolate mofetil; MPA, microscopic polyangiitis; MRC, Medical Research Council Sum Score; MTX, methotrexate; NA, not applicable; ND, not done; NSS, Neurological Symptom Score; NSVN, non-systemic vasculitic neuropathy; RTX, rituximab; sNfL, serum neurofilament light chain; SS, Sjogren's syndrome; SY, symmetric neuropathy
–, Not fulfilled; +, pathologically probable vasculitic neuropathy; ++, pathologically definite vasculitic neuropathy.

Applying an sNfL cut-off value of 155 pg/mL, the sensitivity and specificity to classify correctly between active VN versus VC or NC were 82% and 100%, respectively (area under the receiver operating characteristic curve (AUC) 0.96). There was no difference in sNfL levels between VN at FU and NC, VC or HC (p~0.11).

These data support sNfL as a promising marker to (1) correctly identify patients with VN and to (2) assess VN disease activity, as suggested by the profound decline of sNfL levels during remission. The high specificity of sNfL to axonal damage might be due to the high expression of NfL in large myelinated axons,[4] which are predominantly affected in VN.[10]

This study is limited by its retrospective nature and relatively low number of included individuals. Nevertheless our data suggest sNfL as a biomarker in diagnosis and monitoring of VN and merit further confirmation in a prospective study.

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REFERENCES

1 Collins MP, Dyck PJ, Gronseth GS, *et al*.Peripheral nerve Society Guideline on the classification, diagnosis, investigation, and immunosuppressive therapy of non-systemic vasculitic neuropathy: executive summary. *J Peripher Nerv Syst* 2010;15:176–84.
2 Vrancken AF, Gathier CS, Cats EA, *et al*. The additional yield of combined nerve/muscle biopsy in vasculitic neuropathy. *Eur J Neurol* 2011;18:49–58.
3 Samson M, Puéchal X, Devilliers H, *et al*.Long-term follow-up of a randomized trial on 118 patients with polyarteritis nodosa or microscopic polyangiitis without poor-prognosis factors. *Autoimmun Rev* 2014;13:197–205.
4 Schlaepfer WW, Lynch RG. Immunofluorescence studies of neurofilaments in the rat and human peripheral and central nervous system. *J Cell Biol* 1977;74:241–50.
5 Disanto G, Barro C, Benkert P, *et al*.Serum neurofilament light: a biomarker of neuronal damage in multiple sclerosis. *Ann Neurol* 2017;81:857–70.
6 Steinacker P, Blennow K, Halbgebauer S, *et al*. Neurofilaments in blood and CSF for diagnosis and prediction of onset in Creutzfeldt-Jakob disease. *Sci Rep* 2016;6:38737.
7 Merkies IS, Schmitz PI, van der Meché FG, *et al*.Connecting impairment, disability, and handicap in immune mediated polyneuropathies. *J Neurol Neurosurg Psychiatry* 2003;74:99–104.
8 Dyck PJ, Sherman WR, Hallcher LM, *et al*. Human diabetic endoneurial sorbitol, fructose, and myo-inositol related to sural nerve morphometry. *Ann Neurol* 1980;8:590–6.
9 Suppiah R, Mukhtyar C, Flossmann O, *et al*. A cross-sectional study of the Birmingham Vasculitis Activity score version 3 in systemic vasculitis. *Rheumatology* 2011;50:899–905.
10 Morozumi S, Koike H, Tomita M, *et al*. Spatial distribution of nerve fiber pathology and vasculitis in microscopic polyangiitis-associated neuropathy. *J Neuropathol Exp Neurol* 2011;70:340–8.

Concerns on glucocorticoid use for Japanese patients with established rheumatoid arthritis

I read with great interest the recommendation for the management of rheumatoid arthritis (RA) by the European League Against Rheumatism (EULAR), especially the recommendation regarding glucocorticoid use: 'Short-term glucocorticoids should be considered when initiating or changing conventional synthetic disease-modifying antirheumatic drugs (DMARDs), in different dose regimens and routes of administration, but should be tapered as rapidly as clinically feasible'.[1] This recommendation was primarily supported by randomised controlled studies that suggested the efficacy and acceptable safety of glucocorticoids in early RA compared with placebo.[1 2] However, I am concerned that this recommendation for considering glucocorticoids as an initial strategy for 'early' RA may promote excessive use of glucocorticoids in Japanese patients with 'established' RA.

In Japan, about half of the patients with established RA receive low-dose glucocorticoids.[3–9] A recent cohort study of established patients with RA with a mean disease duration of 7.8 years who initiated treatment with biologic DMARDs showed that glucocorticoid use predicted hospitalised infection with HRs of 3.6 and 1.9 for doses of ≥7.5 mg/day and ≥5 but <7.5 mg/day, respectively.[3] Postmarketing surveillance that included all patients treated with biologics in Japan uniformly showed that glucocorticoid users (prednisolone >5 mg/day) tended to experience serious infection at 2–3 times the frequency of non-users or users of lower dose.[7–9]

A large cohort study of Japanese patients with RA suggested glucocorticoid use as a risk factor for vertebral and non-vertebral bone fractures.[6 10] Furthermore, the risk of mortality increased with increasing prednisolone dose in the same cohort, with HRs of 1.7, 2.4 and 4.2 for doses of 1–5 mg/day, 6–10 mg/day and >10 mg/day, respectively,[5] while dose thresholds associated with mortality were higher in Western countries.[11–13] I think that the predisposition to adverse events related to glucocorticoids in Japanese patients with established RA may partially be explained by the relatively lower weight (mean 53–54 kg).[6 8 9]

The EULAR task force also emphasised that glucocorticoids should be gradually reduced and ultimately stopped, usually within 3 months after starting treatment or by 6 months in exceptional cases.[1] Japanese rheumatologists should also prepare to discontinue glucocorticoids by achieving sufficient control of disease activity with DMARDs to avoid the risks associated with glucocorticoid use, once use of these drugs is started in the management of early RA. Alternatively, we could also consider using intra-articular glucocorticoids or single-dose intravenous/intramuscular glucocorticoids in the initial phase of RA management.[1]

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REFERENCES

1 Smolen JS, Landewé R, Bijlsma J, *et al*. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2016 update. *Ann Rheum Dis* 2017;76:960–77.
2 Smolen JS, Landewé R, Breedveld FC, *et al*. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2013 update. *Ann Rheum Dis* 2014;73:492–509.
3 Mori S, Yoshitama T, Hidaka T, *et al*. Comparative risk of hospitalized infection between biological agents in rheumatoid arthritis patients: A multicenter retrospective cohort study in Japan. *PLoS One* 2017;12:e0179179.
4 Saisho K, Yoshikawa N, Sugata K, *et al*. Prevalence of chronic kidney disease and administration of RA-related drugs in patients with RA: The NinJa 2012 study in Japan. *Mod Rheumatol* 2016;26:331–5.
5 Nakajima A, Inoue E, Tanaka E, *et al*. Mortality and cause of death in Japanese patients with rheumatoid arthritis based on a large observational cohort, IORRA. *Scand J Rheumatol* 2010;39:360–7.
6 Ochi K, Furuya T, Ishibashi M, *et al*. Risk factors associated with the occurrence of proximal humerus fractures in patients with rheumatoid arthritis: a custom strategy for preventing proximal humerus fractures. *Rheumatol Int* 2016;36:213–9.
7 Koike T, Harigai M, Ishiguro N, *et al*. Safety and effectiveness of adalimumab in Japanese rheumatoid arthritis patients: postmarketing surveillance report of 7740 patients. *Mod Rheumatol* 2014;24:390–8.
8 Koike T, Harigai M, Inokuma S, *et al*. Effectiveness and safety of tocilizumab: postmarketing surveillance of 7901 patients with rheumatoid arthritis in Japan. *J Rheumatol* 2014;41:15–23.
9 Harigai M, Ishiguro N, Inokuma S, *et al*. Postmarketing surveillance of the safety and effectiveness of abatacept in Japanese patients with rheumatoid arthritis. *Mod Rheumatol* 2016;26:491–8.
10 Ishida O, Furuya T, Inoue E, *et al*. Risk factors for established vertebral fractures in Japanese patients with rheumatoid arthritis: Results from a large prospective observational cohort study. *Mod Rheumatol* 2015;25:373–8.
11 del Rincón I, Battafarano DF, Restrepo JF, *et al*. Glucocorticoid dose thresholds associated with all-cause and cardiovascular mortality in rheumatoid arthritis. *Arthritis Rheumatol* 2014;66:264–72.
12 Listing J, Kekow J, Manger B, *et al*. Mortality in rheumatoid arthritis: the impact of disease activity, treatment with glucocorticoids, TNFα inhibitors and rituximab. *Ann Rheum Dis* 2015;74:415–21.
13 Movahedi M, Costello R, Lunt M, *et al*. Oral glucocorticoid therapy and all-cause and cause-specific mortality in patients with rheumatoid arthritis: a retrospective cohort study. *Eur J Epidemiol* 2016;31:1045–55.

Response to: 'Concerns on glucocorticoid use for Japanese patients with established rheumatoid arthritis' by Oiwa

We thank Dr Oiwa for the letter discussing the use of glucocorticoids[1] as presented in the most recent update of the European League Against Rheumatism recommendations for the management of rheumatoid arthritis.[2] In light of the Task Force's intensive discussions which were outlined in detail in the text and included safety considerations, we fully agree with the notions made. Therefore, recommendation 6 started with the word 'short-term', and the application of intravenous or intramuscular glucocorticoids was explicitly included when speaking of 'different routes of administration'. In line with Dr Oiwa's conclusion, rheumatologists in individual countries should be prepared to use the best approach to treating RA, possibly with some slight local amendments of our recommendations by national societies.

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REFERENCES

1 Oiwa H. Concerns on glucocorticoid use for Japanese patients with established rheumatoid arthritis. *Ann Rheum Dis* 2018;77:e35.
2 Smolen JS, Landewé R, Bijlsma J, *et al*. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2016 update. *Ann Rheum Dis* 2017;76:960–77.

What is the agreement between pathological features of parotid gland and labial salivary gland biopsies?

We read with a great interest the recent contribution by Mossel *et al*,[1] which brings new important data on the diagnostic value of major salivary ultrasonography (SGUS) for primary Sjögren's syndrome (pSS), as reported in previous studies[2–10] and stresses the need to evaluate the inclusion of SGUS into future classification criteria for the disease.

In their cohort of 103 consecutive patients clinically suspected for pSS, Mossel *et al* reported a good agreement between SGUS and labial and parotid salivary gland biopsy. Agreement was reported to be marginally higher for parotid gland biopsy (absolute agreement of 83%) than for minor labial salivary gland biopsy (absolute agreement 79%). However, the ability of SGUS to predict a positive biopsy was different for labial and parotid biopsies. The positive predictive value (PPV) and negative predictive value (NPV) of SGUS to predict the parotid biopsy result were, respectively, 78% and 86%, whereas the PPV and NPV of SGUS to predict a labial salivary gland result were, respectively, 84% and 74%. Furthermore, the best cut-off to define a positive SGUS was different if the outcome of interest was parotid or labial biopsy results. Altogether, these observations suggest that the labial and parotid biopsy results are not always concordant.

Since the description of abnormal minor salivary gland biopsy in patients with Sjögren's syndrome by Chisholm and Mason in 1968,[11] this method has been preferred to major salivary gland biopsy by most groups in clinical practice,[12] in clinical trials[13] and in the different classification criteria.[14–17] In their subsequent work, published in 1970, Chisholm *et al* formally compared labial and major salivary gland biopsies in a large series of 116 postmortem subjects and reported that, while focal lymphocytic adenitis was present in the major salivary glands in a significant proportion of the subjects, none of them had focus formation in the labial glands salivary glands, suggesting a better specificity of the latter procedure for Sjögren's syndrome.[18] Recent guidelines on the standardisation of salivary gland biopsy focused on labial salivary gland.[19] In a previous smaller study, investigators from the same group reported a good agreement between parotid and labial salivary gland biopsy for the diagnosis of pSS.[20] Could the authors precise the agreement between these two procedures, in the 43 patients who had both labial and parotid biopsies in their most recent work?

Besides its diagnostic value, salivary gland biopsy may also give useful information to the clinicians, in terms of prognostic including risk of lymphoma or response to therapy.[21–27] In that respect, a quantitative analysis of the degree of lymphocytic infiltrate may be necessary. It would be highly interesting to know what was the correlation between the focus score evaluated on the labial biopsy and the focus score evaluated on the parotid biopsy, in patients who underwent the two procedures.

Finally, we previously reported that the correlation between SGUS score and the focus score on labial salivary gland biopsy was high (Spearman $r=0.61$, $p<0.001$).[24] Such analyses, considering the focus score as a quantitative outcome, would be also interesting to compare with SGUS in both type of biopsy in the current study.

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REFERENCES

1 Mossel E, Delli K, van Nimwegen JF, *et al*. Ultrasonography of major salivary glands compared with parotid and labial gland biopsy and classification criteria in patients with clinically suspected primary Sjögren's syndrome. *Ann Rheum Dis* 2017;76:1883–9.
2 Jousse-Joulin S, Milic V, Jonsson MV, *et al*. Is salivary gland ultrasonography a useful tool in Sjögren's syndrome? A systematic review. *Rheumatology* 2016;55:789–800.
3 Cornec D, Jousse-Joulin S, Pers JO, *et al*. Contribution of salivary gland ultrasonography to the diagnosis of Sjögren's syndrome: toward new diagnostic criteria? *Arthritis Rheum* 2013;65:216–25.
4 Theander E, Mandl T. Primary Sjögren's syndrome: diagnostic and prognostic value of salivary gland ultrasonography using a simplified scoring system. *Arthritis Care Res* 2014;66:1102–7.
5 Cornec D, Jousse-Joulin S, Marhadour T, *et al*. Salivary gland ultrasonography improves the diagnostic performance of the 2012 American College of Rheumatology classification criteria for Sjögren's syndrome. *Rheumatology* 2014;53:1604–7.
6 Zhang X, Zhang S, He J, *et al*. Ultrasonographic evaluation of major salivary glands in primary Sjögren's syndrome: comparison of two scoring systems. *Rheumatology* 2015;54:1680–7.
7 Luciano N, Baldini C, Tarantini G, *et al*. Ultrasonography of major salivary glands: a highly specific tool for distinguishing primary Sjögren's syndrome from undifferentiated connective tissue diseases. *Rheumatology* 2015;54:kev253–204.
8 Baldini C, Luciano N, Tarantini G, *et al*. Salivary gland ultrasonography: a highly specific tool for the early diagnosis of primary Sjögren's syndrome. *Arthritis Res Ther* 2015;17:146.
9 Hammenfors DS, Brun JG, Jonsson R, *et al*. Diagnostic utility of major salivary gland ultrasonography in primary Sjögren's syndrome. *Clin Exp Rheumatol* 2015;33:56–62.
10 Gazeau P, Cornec D, Jousse-Joulin S, *et al*. Time-course of ultrasound abnormalities of major salivary glands in suspected Sjögren's syndrome. *Joint Bone Spine* 2017 (Epub ahead of print 27 Feb 2017).
11 Chisholm DM, Mason DK. Labial salivary gland biopsy in Sjögren's disease. *J Clin Pathol* 1968;21:656–60.
12 Guellec D, Cornec D, Jousse-Joulin S, *et al*. Diagnostic value of labial minor salivary gland biopsy for Sjögren's syndrome: a systematic review. *Autoimmun Rev* 2013;12:416–20.
13 Costa S, Quintin-Roué I, Lesourd A, *et al*. Reliability of histopathological salivary gland biopsy assessment in Sjögren's syndrome: a multicentre cohort study. *Rheumatology* 2015;54:1056–64.

14 Vitali C, Bombardieri S, Jonsson R, *et al*. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis* 2002;61:554–8.
15 Shiboski SC, Shiboski CH, Criswell L, *et al*. American College of Rheumatology classification criteria for Sjögren's syndrome: a data-driven, expert consensus approach in the Sjögren's International Collaborative Clinical Alliance cohort. *Arthritis Care Res* 2012;64:475–87.
16 Shiboski CH, Shiboski SC, Seror R, *et al*. American college of rheumatology/European league against rheumatism classification criteria for primary Sjögren's Syndrome: a consensus and data-driven methodology involving three international patient cohorts. *Arthritis Rheumatol Hoboken NJ* 2016;2017:35–45.
17 Shiboski CH, Shiboski SC, Seror R, *et al*. American college of rheumatology/European league against rheumatism classification criteria for primary Sjögren's syndrome: a consensus and data-driven methodology involving three international patient cohorts. *Ann Rheum Dis* 2016;2017:9–16.
18 Chisholm DM, Waterhouse JP, Mason DK. Lymphocytic sialadenitis in the major and minor glands: a correlation in postmortem subjects. *J Clin Pathol* 1970;23:690–4.
19 Fisher BA, Jonsson R, Daniels T, *et al*. Standardisation of labial salivary gland histopathology in clinical trials in primary Sjögren's syndrome. *Ann Rheum Dis* 2017;76:1161–8.
20 Pijpe J, Kalk WW, van der Wal JE, *et al*. Parotid gland biopsy compared with labial biopsy in the diagnosis of patients with primary Sjogren's syndrome. *Rheumatology* 2007;46:335–41.
21 Fisher BA, Brown RM, Bowman SJ, *et al*. A review of salivary gland histopathology in primary Sjögren's syndrome with a focus on its potential as a clinical trials biomarker. *Ann Rheum Dis* 2015;74:1645–50.
22 Carubbi F, Alunno A, Cipriani P, *et al*. Is minor salivary gland biopsy more than a diagnostic tool in primary Sjögren's syndrome? Association between clinical, histopathological, and molecular features: a retrospective study. *Semin Arthritis Rheum* 2014;44:314–24.
23 Risselada AP, Kruize AA, Goldschmeding R, *et al*. The prognostic value of routinely performed minor salivary gland assessments in primary Sjögren's syndrome. *Ann Rheum Dis* 2014;73:1537–40.
24 Cornec D, Jousse-Joulin S, Costa S, *et al*. High-grade salivary-gland involvement, assessed by histology or ultrasonography, is associated with a poor response to a single rituximab course in primary Sjögren's syndrome: data from the TEARS randomized trial. *PLoS One* 2016;11:e0162787.
25 Cornec D, Costa S, Devauchelle-Pensec V, *et al*. Blood and salivary-gland BAFF-driven B-cell hyperactivity is associated to rituximab inefficacy in primary Sjögren's syndrome. *J Autoimmun* 2016;67:102–10.
26 Delli K, Haacke EA, Kroese FG, *et al*. Towards personalised treatment in primary Sjögren's syndrome: baseline parotid histopathology predicts responsiveness to rituximab treatment. *Ann Rheum Dis* 2016;75:1933–8.
27 Cornec D, Costa S, Devauchelle-Pensec V, *et al*. Do high numbers of salivary gland-infiltrating B cells predict better or worse outcomes after rituximab in patients with primary Sjögren's syndrome? *Ann Rheum Dis* 2016;75:e33.

The parotid gland connection: ultrasound and biopsies in primary Sjögren's syndrome

We would like to thank Alegria *et al*[1] for their letter to the editor commenting on our recent publication entitled 'Ultrasonography of major salivary glands compared with parotid and labial gland biopsy and classification criteria in patients with clinically suspected primary Sjögren's syndrome'.[2] Our study was the first that directly compared the validity of ultrasound of major salivary glands (sUS) with parotid gland biopsy outcome. We showed that the agreement between sUS and parotid as well as labial gland biopsies was good but was slightly higher for the former.

As noted by Alegria *et al*,[1] we found different results for the positive predictive value (PPV) and negative predictive value (NPV) between sUS versus parotid and sUS versus labial gland biopsies.[2] Although these observations may indeed be caused by discordance between the parotid and labial gland biopsy outcome, there are other factors that may equally well contribute to this discrepancy. First, the parotid glands are assessed during the sUS examination and included in the scoring, whereas the labial glands are not.[2] Second, in 6%–15% of the general population, the labial gland biopsy is positive, while in only 5% of the general population, the parotid gland biopsy is positive.[3–6] This implies that when biopsies of parotid and labial glands are taken simultaneously, at least up to 10% of the biopsies may be discordant. Third, the patient cohort in which sUS was compared with parotid gland biopsies was not exactly the same as the patient cohort in which sUS was compared with labial gland biopsies. However, when only taking into account the 43 patients who underwent both a parotid and labial gland biopsy, there still was a discrepancy between the predictive values of parotid and labial gland biopsies. In this group of 43 patients, compared with parotid gland biopsies, sUS showed a PPV of 64% (7/11) and NPV of 88% (28/32), and when compared with labial gland biopsies, PPV was 73% (11/15) and NPV was 75% (21/28).

Alegria *et al*[1] requested more information about the group of 43 patients who had both a parotid and labial gland biopsy, that is, the agreement between both biopsies and the correlation between sUS and the focus score in the parotid and labial gland biopsies. We fully agree with Alegria *et al*[1] that it is important to make a direct comparison between parotid and labial salivary gland biopsies and not only to compare both to sUS. Such a detailed comparison of the results obtained with parotid and labial gland biopsies is currently in progress in a larger cohort of patients. In this study, the focus score of the parotid and labial gland biopsies will be compared, as suggested by Alegria *et al*, and the other histopathological characteristics of primary Sjögren's syndrome in both types of biopsies.

In the current study, in the 43 patients who underwent a double biopsy, the correlation between sUS and parotid gland focus score was ρ=0.376 (figure 1A), while the correlation between sUS and labial gland focus score was ρ=0.412 (figure 1B). Previously, Cornec *et al* reported a correlation of ρ=0.61 between sUS and focus score in labial salivary glands of participants in the TEARS trial.[7] However, all patients in their cohort were already classified as primary Sjögren's syndrome according to the American European Consensus Group criteria, in contrast to our study, in which patients clinically suspected with p were included.[7 8] This difference in patient selection could be the explanation for the discrepancy in reported correlation.

As Alegria *et al*[1] stated, Chisholm *et al*[9] found that minor salivary gland biopsies have a greater specificity compared with major salivary gland biopsies. Whether this study is representative for current medical practice is discussable. First, Chisholm *et al*[9] evaluated major salivary gland biopsies from the submandibular gland, whereas in our study,[2] parotid gland biopsies were evaluated. Whether the histopathology of the submandibular gland is similar to the parotid gland is unknown, since submandibular salivary glands are not easily accessible for taking incisional biopsies. Second, another important difference is that Chisholm *et al*[9] performed a postmortem study. Thus, the study of Chisholm *et al*[9] cannot be used as a direct comparison. On the other hand, two other studies showed that major salivary gland biopsies performed at least as well as minor salivary gland biopsies in the diagnosis of primary Sjögren's syndrome.[6 10]

Currently, most groups still prefer to perform labial gland instead of parotid gland biopsies, as taking parotid gland biopsies requires specific surgical expertise.[6] Therefore, labial gland biopsies are included in the various classification criteria sets and were the main focus of recent guidelines, regarding the histopathological evaluation of Sjögren's syndrome.[8 11–13] However, there are several advantages of parotid gland biopsies

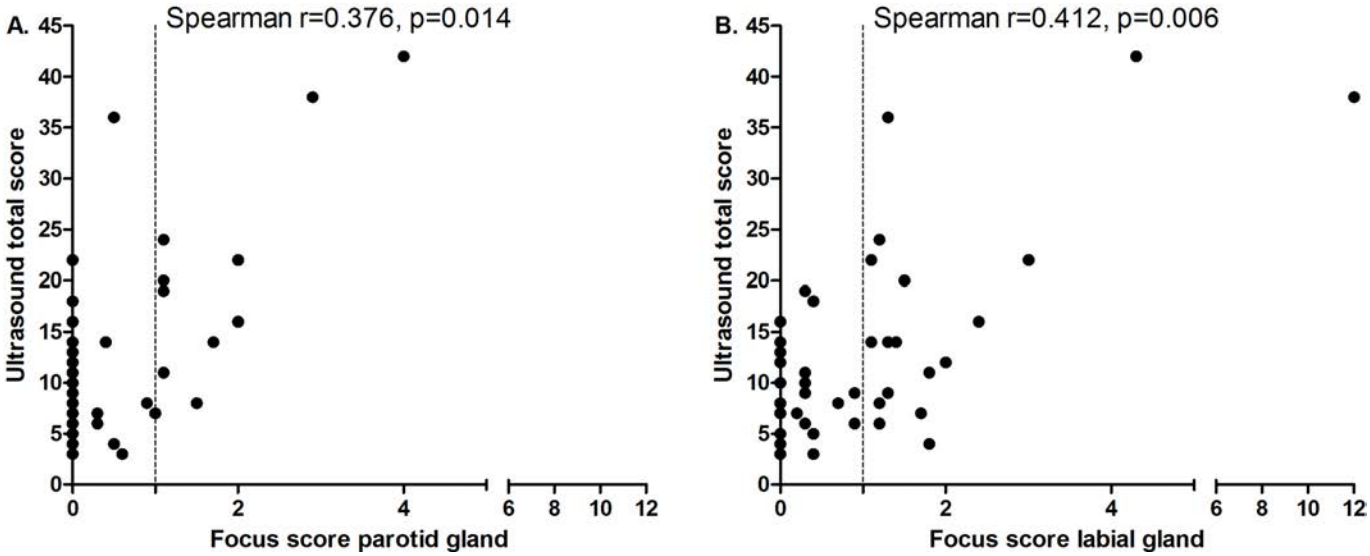

Figure 1 (A) Ultrasound total score compared with focus score in parotid gland biopsies. (B) Ultrasound total score compared with focus score in labial gland biopsies.

in comparison to labial gland biopsies, including the possibility of identifying mucosa-associated lymphoid tissue lymphoma at an earlier stage, the possibility of performing repeated biopsies of the same gland and allowing a direct gland-specific comparison with other diagnostic methods, like glandular specific saliva and ultrasound.[6 14 15] Furthermore, in the study of Marx *et al*,[10] in five Sjögren's syndrome patients, a diagnosis of lymphoma was made based on the parotid gland biopsies, while the labial gland biopsies all lacked lymphomatous changes. Interestingly, we have shown that the baseline number of $CD20^+$ B-cells/mm^2 of parenchyma could serve as a prognostic biomarker to predict rituximab response. Hence, baseline characteristics of the parotid gland biopsy might be our guide to personalised treatment.[16]

To conclude, the parotid gland should not be ignored and is an important organ in the evaluation of Sjögren's syndrome.

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REFERENCES

1 Alegria GC, Costa S, Jousse-Joulin S, *et al*. What is the agreement between pathological features of parotid gland and labial salivary gland biopsies? *Ann Rheum Dis* 2017.
2 Mossel E, Delli K, van Nimwegen JF, *et al*. Ultrasonography of major salivary glands compared with parotid and labial gland biopsy and classification criteria in patients with clinically suspected primary Sjögren's syndrome. *Ann Rheum Dis* 2017:annrheumdis-2017-211250.
3 Daniels TE. Labial salivary gland biopsy in Sjögren's syndrome. Assessment as a diagnostic criterion in 362 suspected cases. *Arthritis Rheum* 1984;27:147–56.
4 Segerberg-Konttinen M, Konttinen YT, Bergroth V. Focus score in the diagnosis of Sjögren's syndrome. *Scand J Rheumatol Suppl* 1986;61:47–51.
5 Radfar L, Kleiner DE, Fox PC, *et al*. Prevalence and clinical significance of lymphocytic foci in minor salivary glands of healthy volunteers. *Arthritis Rheum* 2002;47:520–4.
6 Pijpe J, Kalk WW, van der Wal JE, *et al*. Parotid gland biopsy compared with labial biopsy in the diagnosis of patients with primary Sjogren's syndrome. *Rheumatology* 2007;46:335–41.
7 Cornec D, Jousse-Joulin S, Costa S, *et al*. High-grade salivary-gland involvement, assessed by histology or ultrasonography, is associated with a poor response to a single rituximab course in primary Sjögren's syndrome: data from the TEARS randomized trial. *PLoS One* 2016;11:e0162787–11.
8 Vitali C, Bombardieri S, Jonsson R, *et al*. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis* 2002;61:554–8.
9 Chisholm DM, Waterhouse JP, Mason DK. Lymphocytic sialadenitis in the major and minor glands: a correlation in postmortem subjects. *J Clin Pathol* 1970;23:690–4.
10 Marx RE, Hartman KS, Rethman KV. A prospective study comparing incisional labial to incisional parotid biopsies in the detection and confirmation of sarcoidosis, Sjögren's disease, sialosis and lymphoma. *J Rheumatol* 1988;15:621–9.
11 Shiboski SC, Shiboski CH, Criswell L, *et al*. American college of rheumatology classification criteria for Sjögren's syndrome: a data-driven, expert consensus approach in the Sjögren's international collaborative clinical alliance cohort. *Arthritis Care Res* 2012;64:475–87.
12 Shiboski CH, Shiboski SC, Seror R, *et al*. American College of Rheumatology/European League Against Rheumatism classification criteria for primary Sjögren's syndrome. *Ann Rheum Dis* 2016;2017:9–16.
13 Fisher BA, Jonsson R, Daniels T, *et al*. Sjögren's histopathology workshop group (appendix) from ESSENTIAL (EULAR Sjögren's syndrome study group). Standardisation of labial salivary gland histopathology in clinical trials in primary Sjögren's syndrome. *Ann Rheum Dis* 2017;76:1161–8.
14 Spijkervet FK, Haacke E, Kroese FG, *et al*. Parotid gland biopsy, the alternative way to diagnose Sjögren syndrome. *Rheum Dis Clin North Am* 2016;42:485–99.
15 Delli K, Vissink A, Spijkervet FK. Salivary gland biopsy for Sjögren's syndrome. *Oral Maxillofac Surg Clin North Am* 2014;26:23–33.
16 Delli K, Haacke EA, Kroese FG, *et al*. Towards personalised treatment in primary Sjögren's syndrome: baseline parotid histopathology predicts responsiveness to rituximab treatment. *Ann Rheum Dis* 2016;75:1933–8.

Different glucosamine sulfate products generate different outcomes on osteoarthritis symptoms

Runhaar and colleagues[1] produced an elegant meta-analysis of various glucosamine-containing products in knee and hip osteoarthritis (OA), based on individual patient data (IPD). Although they were able to access and analyse only 5 out 21 eligible studies, their results are in agreement with most glucosamine meta-analyses in OA: glucosamine products other than prescription crystalline glucosamine sulfate are not effective in hip or knee OA pain and function.

Such results, as acknowledged by the Authors, had already been demonstrated in several previous and more comprehensive meta-analyses, including a dedicated Cochrane Review[2] and a recent effort by Eriksen *et al*[3] specifically investigating possible differences in efficacy among glucosamine products. Indeed, glucosamine exists in different forms for pharmaceutical use, as extensively reviewed by Altman.[4] Among these, glucosamine hydrochloride (used in three out of five studies in the Runhaar *et al* IPD meta-analysis) is the most readily available glucosamine salt, but a certified ineffective product since the 2010 edition of the Osteoarthritis Research Society International OA treatment recommendations,[5] mainly because of its pharmacokinetics limitations.[4] On the other hand, glucosamine sulfate is a hygroscopic compound that must be stabilised: this is appropriately done in crystalline glucosamine sulfate only and by a patented method.[6] In fact, this compound is approved as a prescription drug in Europe and elsewhere.

Actually, other putative 'glucosamine sulfate' products (two studies with non-characterised 'glucosamine sulfate' products in the Runhaar *et al* IPD meta-analysis) have been widely shown ineffective, at variance with crystalline glucosamine sulfate.[2 3] The leading group of the present OA Trial Bank initiative[1] is well aware of this, as acknowledged in their article and based on a previous study of theirs in which crystalline glucosamine sulfate even showed compelling hints of efficacy in an OA prevention trial setting.[7] Nevertheless, they were denied access to the three large and high-quality (ie, low risk of bias) pivotal trial data of prescription crystalline glucosamine sulfate[8–10] by the sponsor (Rottapharm). This was probably because two different meta-analyses[3 11] had already produced identical and favourable results of such studies, mirroring the conclusions from the Cochrane Review.[2] It was felt perhaps inappropriate then, to pool the outcomes of such trials with those of ineffective products that have not been characterised in the same way in terms of quality,[4] pharmacokinetics[12] and correspondence of human biological fluid levels with mechanistic data,[13] irrespective of any possible secondary subgroup analysis. Thus, while data sharing from clinical trials should be encouraged, sponsor should also be reassured about the use of such data, especially when completely different products are considered in meta-analyses. Finally, Runhaar *et al* could not find predictors of response in negative studies of non-characterised glucosamine products by the IPD technique, as we were not able to find such predictors in favourable trials of crystalline glucosamine sulfate by other techniques[14–16] : this point remains therefore unanswered.

In conclusion, the data by Runhaar and colleagues[1] confirm that glucosamine hydrochloride and non-characterised glucosamine sulfate products are ineffective in OA. Indeed, the recent recommendations by the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases strongly support that only prescription crystalline glucosamine sulfate should be used as background pharmacological treatment in the first step of the knee OA management algorithm,[17] since other glucosamine-containing products are not effective as confirmed by this meta-analysis.[1]

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REFERENCES

1 Runhaar J, Rozendaal RM, van Middelkoop MV, *et al*. Subgroup analyses of the effectiveness of oral glucosamine for knee and hip osteoarthritis: a systematic review and individual patient data meta-analysis from the OA trial bank. *Ann Rheum Dis* 2017;76:1862–9.
2 Towheed T, Maxwell L, Anastassiades TP, *et al*. Glucosamine therapy for treating osteoarthritis. *Cochrane Database Syst Rev* 2009;18:CD002946.
3 Eriksen P, Bartels EM, Altman RD, *et al*. Risk of bias and brand explain the observed inconsistency in trials on glucosamine for symptomatic relief of osteoarthritis: a meta-analysis of placebo-controlled trials. *Arthritis Care Res* 2014;66:1844–55.
4 Altman RD. Glucosamine therapy for knee osteoarthritis: pharmacokinetic considerations. *Expert Rev Clin Pharmacol* 2009;2:359–71.
5 Zhang W, Nuki G, Moskowitz RW, *et al*. OARSI recommendations for the management of hip and knee osteoarthritis: part III: Changes in evidence following systematic cumulative update of research published through January 2009. *Osteoarthritis Cartilage* 2010;18:476–99.
6 De Wan M, Volpi G. inventors. A method of preparing mixed glucosamine salts. *US patent* 1997;5:107.
7 Runhaar J, Deroisy R, van Middelkoop M, *et al*. The role of diet and exercise and of glucosamine sulfate in the prevention of knee osteoarthritis: Further results from the PRevention of knee Osteoarthritis in Overweight Females (PROOF) study. *Semin Arthritis Rheum* 2016;45(4 Suppl):S42–S48.
8 Reginster JY, Deroisy R, Rovati LC, *et al*. Long-term effects of glucosamine sulphate on osteoarthritis progression: a randomised, placebo-controlled clinical trial. *Lancet* 2001;357:251–6.
9 Pavelká K, Gatterová J, Olejarová M, *et al*. Glucosamine sulfate use and delay of progression of knee osteoarthritis: a 3-year, randomized, placebo-controlled, double-blind study. *Arch Intern Med* 2002;162:2113–23.
10 Herrero-Beaumont G, Ivorra JA, Del Carmen Trabado M, *et al*. Glucosamine sulfate in the treatment of knee osteoarthritis symptoms: a randomized, double-blind, placebo-controlled study using acetaminophen as a side comparator. *Arthritis Rheum* 2007;56:555–67.

11 Reginster JY. The efficacy of glucosamine sulfate in osteoarthritis: financial and nonfinancial conflict of interest. *Arthritis Rheum* 2007;56:2105–10.
12 Persiani S, Rotini R, Trisolino G, *et al*. Synovial and plasma glucosamine concentrations in osteoarthritic patients following oral crystalline glucosamine sulphate at therapeutic dose. *Osteoarthritis Cartilage* 2007;15:764–72.
13 Chiusaroli R, Piepoli T, Zanelli T, *et al*. Experimental pharmacology of glucosamine sulfate. *Int J Rheumatol* 2011;2011:1–8.
14 Bruyere O, Honore A, Rovati LC, *et al*. Radiologic features poorly predict clinical outcomes in knee osteoarthritis. *Scand J Rheumatol* 2002;31:13–16.
15 Bruyere O, Honore A, Ethgen O, *et al*. Correlation between radiographic severity of knee osteoarthritis and future disease progression. Results from a 3-year prospective, placebo-controlled study evaluating the effect of glucosamine sulfate. *Osteoarthritis Cartilage* 2003;11:1–5.
16 Pavelka K, Bruyere O, Rovati LC, *et al*. Relief in mild-to-moderate pain is not a confounder in joint space narrowing assessment of full extension knee radiographs in recent osteoarthritis structure-modifying drug trials. *Osteoarthritis Cartilage* 2003;11:730–7.
17 Bruyère O, Cooper C, Pelletier JP, *et al*. An algorithm recommendation for the management of knee osteoarthritis in Europe and internationally: a report from a task force of the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO). *Semin Arthritis Rheum* 2014;44:253–63.

Response to: 'Different glucosamine sulfate products generate different outcomes on osteoarthritis symptoms' by Reginster *et al*

We appreciate the interest that Reginster and colleagues[1] showed in our publication on subgroup effects of oral glucosamine for osteoarthritis (OA).[2] We are pleased that the eLetter authors do not put forward any critique of our publication that requires our justification or clarification.

As highlighted by the authors,[1] the effectiveness of oral glucosamine products for knee and hip OA symptoms has been questioned. Nevertheless, due to the heterogeneous nature of the disease, whether certain subgroups of patients with OA could benefit from glucosamine has not been excluded. Since available randomised controlled trials are usually not powered to show subgroup effects, these effects might have been overlooked before. We therefore undertook this individual patient data meta-analysis,[2] where more than 50% of all available participants (1625 individuals) from the placebo-controlled trials were retrieved to evaluate the effectiveness of oral glucosamine for several predefined clinical relevant subgroups, using methodologically robust methods. We would highlight that, contrary to what Reginster *et al* suggest, the presented results (ie, oral glucosamine was not superior to placebo in any of the predefined subgroups) do add to the available knowledge regarding the effectiveness of oral glucosamine for OA, as analyses within clinical relevant subgroups using individual patient data have not been published before.

In their eLetter, Reginster *et al* suggest that different glucosamine compounds may have different effectiveness for OA symptoms. Since we were fully aware of this, our analyses plan, as designed and shared with trial owners when inviting for data sharing, included stratified analyses for different glucosamine compounds. Despite these intentions to properly address the potential of differences in effectiveness for different glucosamine compounds, the request for data sharing was declined by the study owner of the crystalline glucosamine sulfate trials.

We are pleased to note that Reginster and colleagues endorse our call for open data. Unfortunately, open trial data is not yet a common practice for clinical trials. The lack of open trial data limits the interpretation of clinical trial reports, especially when those trials were performed with significant industry sponsor involvement, thus jeopardising the appropriateness of recommendations for clinical decision-making.[3] Therefore, some major international funding bodies now require open data access for studies that they fund. Given the special attention paid in our analysis plan to potential differences in effectiveness for different glucosamine compounds, the negative reply by the study owner raises concerns about the robustness of their study findings. Obviously, this is also a concern for non-industry trials that were not shared with the OA Trial Bank.

Together with Reginster and colleagues, in the interest of determining the optimal treatment for people with OA, we strongly encourage open discussions on the benefits and barriers to open trial data among all involved parties. This is in line with the call from the Institution of Medicine on 'sharing individual patient data from clinical trials'.[4]

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Correction notice This article has been corrected since it published Online First. The third author's name has been corrected to Marienke van Middelkoop.

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REFERENCES

1 Reginster JL, Bruyere O, Cooper C. Different glucosamine sulfate products generate different outcomes on osteoarthritis symptoms. *Ann Rheum Dis* 2018:e39.
2 Runhaar J, Rozendaal RM, van Middelkoop M, *et al*. Subgroup analyses of the effectiveness of oral glucosamine for knee and hip osteoarthritis: a systematic review and individual patient data meta-analysis from the OA trial bank. *Ann Rheum Dis* 2017;76:1862–9.
3 Fleetcroft R, Ford J, Gollop ND, *et al*. Difficulty accessing data from randomised trials of drugs for heart failure: a call for action. *BMJ* 2015;351:h5002.
4 Drazen JM. Sharing individual patient data from clinical trials. *N Engl J Med Overseas Ed* 2015;372:201–2.

Lesinurad combination therapy with allopurinol in gout: do CLEAR studies make the treatment of gout clearer?

In his recent editorial commenting on the Combining Lesinurad with Allopurinol in Inadequate Responders studies,[1] Dr Singh provides a thorough overview of the phase 3 trials demonstrating the efficacy and safety of lesinurad given in combination with allopurinol for the treatment of gout in patients who do not reach serum uric acid goal on allopurinol alone. We would like to thank Dr Singh for his thoughtful and balanced commentary. We would like to point out that in his comments Dr Singh advises the need to cautiously evaluate and discuss the risk/benefit ratio of lesinurad 400 mg given in combination with allopurinol in patients without normal renal function because of the higher risk of serious adverse events and renal adverse events. Although lesinurad was studied at both the 200 mg and 400 mg dose given in combination with allopurinol,[2 3] there was a higher incidence of serum creatinine elevations and renal-related adverse reactions observed with the lesinurad 400 mg dose compared with the lesinurad 200 mg dose. Based on these findings, the sponsor did not pursue regulatory approval for the 400 mg dose. Therefore, lesinurad 200 mg is the only (initial and maximum) dose that should be prescribed to patients in combination with a xanthine oxidase inhibitor in those who do not reach target serum uric acid goal with a xanthine oxidase inhibitor alone.

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REFERENCES

1 Singh JA. Lesinurad combination therapy with allopurinol in gout: do CLEAR studies make the treatment of gout clearer? *Ann Rheum Dis* 2017;76:779–81.
2 Saag K, Fitz-Patrick D, Kopicko J, *et al*. Lesinurad combined with allopurinol: randomized, double-blind, placebo-controlled study in gout subjects with inadequate response to standard of care allopurinol (a US-based study). *Arthritis Rheum* 2016.
3 Bardin T, Keenan RT, Khanna PP, *et al*. Lesinurad in combination with allopurinol: a randomised, double-blind, placebo-controlled study in patients with gout with inadequate response to standard of care (the multinational CLEAR 2 study). *Ann Rheum Dis* 2017;76:811–20.

Response to: 'A response to Singh. "Lesinurad combination therapy with allopurinol in gout: do CLEAR studies make the treatment of gout clearer?"' by Valiyil and Schechter

I thank Valiyil and Schechter for their letter[1] to the editorial related to clinical studies of lesinurad.[2] The letter correctly states that the 200 mg lesinurad dose is the only (initial and maximum) dose that should be prescribed to patients, always in combination with a xanthine oxidase inhibitor. The letter provides an important clarification regarding the dose of lesinurad that was approved by the regulatory agencies. Based on higher toxicity, the sponsor did not pursue regulatory approval (Food and Drug Administration (FDA) and European Medicines Agency (EMEA)) for the 400 mg lesinurad dose in combination with a xanthine oxidase inhibitor. In the editorial,[2] I offered caution and the need for careful monitoring when considering the use of lesinurad at the higher dose, that is, 400 mg in combination with xanthine oxidase inhibitor.[3 4] Although not approved by the regulatory agencies, such use might rarely occur in clinical practice, based on an individual's situation. The existing evidence of higher renal toxicity with the higher lesinurad dose[3 4] is a reason to consider other options for urate-lowering, such as pegloticase or combining xanthine oxidase with other uricosurics such as probenecid, if patients fail to respond to lesinurad 200 mg in combination with a xanthine oxidase inhibitor. Such an approach may be safer. A bigger potential risk is the use of lesinurad alone (without xanthine oxidase inhibitor), which should not be done. The recent FDA approval of a combination pill consisting of 200 mg lesinurad plus allopurinol 300 mg should help prevent that error to some extent,[5] but a concerted educational effort by the manufacturer may be needed to further ensure an appropriate use of lesinurad. An effective, safe use of urate-lowering therapies is key to optimal gout management, including the achievement of target serum urate, which in turn has well-known long-term benefits.

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Competing interests JAS has received research grants from Takeda and Savient and consultant fees from Savient, Takeda, Regeneron, Merz, Iroko, Bioiberica, Crealta/Horizon and Allergan pharmaceuticals, WebMD, UBM LLC and the American College of Rheumatology. JAS serves as the principal investigator for an investigator-initiated study funded by Horizon pharmaceuticals through a grant to DINORA, Inc, a 501 (c)(3) entity. JAS is a member of the executive of OMERACT, an organisation that develops outcome measures in rheumatology and receives arms-length funding from 36 companies; a member of the American College of Rheumatology's (ACR) Annual Meeting Planning Committee (AMPC); Chair of the ACR Meet-the-Professor, Workshop and Study Group Subcommittee; and a member of the Veterans Affairs Rheumatology Field Advisory Committee. JAS is the editor and the Director of the UAB Cochrane Musculoskeletal Group Satellite Center on Network Meta-analysis.

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REFERENCES

1 Valiyil R, Schechter B. A response to Singh. "Lesinurad combination therapy with allopurinol in gout: do CLEAR studies make the treatment of gout clearer?". *Ann Rheum Dis* 2017.
2 Singh JA. Lesinurad combination therapy with allopurinol in gout: do CLEAR studies make the treatment of gout clearer? *Ann Rheum Dis* 2017;76:779–81.
3 Bardin T, Keenan RT, Khanna PP, *et al*. Lesinurad in combination with allopurinol: a randomised, double-blind, placebo-controlled study in patients with gout with inadequate response to standard of care (the multinational CLEAR 2 study). *Ann Rheum Dis* 2017;76:811–20.
4 Saag KG, Fitz-Patrick D, Kopicko J, *et al*. Lesinurad combined with allopurinol: a randomized, double-blind, placebo-controlled study in gout patients with an inadequate response to standard-of-care allopurinol (a US-based study). *Arthritis Rheumatol* 2017;69:203–12.
5 A Bershire Hathaway Company. Ironwood pharmaceuticals announces FDA approval of DUZALLO® (lesinurad and allopurinol) for the treatment of hyperuricemia in patients with uncontrolled gout. http://www.businesswire.com/news/home/20170821005414/en/Ironwood-Pharmaceuticals-Announces-FDA-Approval-DUZALLO%C2%AE-lesinurad.

Correction

Based on information provided by the World Health Organization (WHO), the authors wish to correct statements implying that WHO is associated with or endorses the FRAX® model or treatment recommendations of at-risk populations. The FRAX® tool was not developed, endorsed, evaluated or validated by WHO

10.1136/annrheumdis-2012-eular.1026

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Correction

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10.1136/annrheumdis-2016-eular.4694

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Correction

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EXTENDED REPORT

Retracted: Methotrexate in the treatment of symptomatic knee osteoarthritis: randomised placebo-controlled trial

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ABSTRACT

Objectives To assess the efficacy of methotrexate (MTX) in decreasing pain and inflammation in symptomatic knee osteoarthritis (OA).

Methods One hundred and forty-four patients with primary knee OA were randomised in a 1:1 ratio to receive up to 25 mg/week oral MTX (n=72) or placebo (n=72) for 28 weeks. Outcome measures included reduction in pain and inflammation and improvements in physical function scores. Pain was assessed using the visual analogue pain scale, (VAS, 0–100 mm). Functional assessment was performed using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and activities of daily living (ADL) scores. Synovitis was detected clinically and by ultrasound imaging at baseline and at the end of the study.

Results There was a clinically relevant reduction in the intervention group compared with the placebo group for knee pain, physical function and ADL scores at 28 weeks. The mean difference between treatment arms (95% CI) was 11.4 (2.8 to 20.0), p=0.009; 9.5 (3.7 to 15.3) p=0.001 and 1.2 (0.1 to 2.3), p=0.032, respectively. Furthermore, a clinically relevant reduction in synovitis (both clinically and by ultrasound) was noted in the MTX group compared with the placebo group at 28 weeks. The proportion of patients who had a reduction in VAS of >20 mm was significantly higher in the MTX group (n=38) 53% than in the placebo group (n=17) 24%, p=0.018.

Conclusions MTX significantly reduced pain and improved synovitis. There was a significant improvement in physical function. MTX may be a therapeutic option in the treatment of pain and inflammation related to knee OA.

Trial registration number: NCT01927484

INTRODUCTION

Osteoarthritis (OA) is a progressive complex, multifactorial disease that affects all joint structures, with patients classified as heterogeneous groups, exhibiting varying degrees of inflammation, in some cases comparable with rheumatoid arthritis. The precise aetiology remains unclear. Inflammation has been implicated in the pathogenesis of OA and may be either a primary event or secondary to other aspects of the disease, such as biochemical changes within the cartilage.[1–3]

Synovial inflammation and proliferation is a key component of OA and a predictor of worsening disease. Synovial inflammation due to the release of prostaglandins and cytokines is an important cause of pain.[3–5] Knee pain, the leading symptom of knee OA, is often chronic, leading to significant morbidity and disability.[1]

Thus, for clinical management, pain reduction and functional improvement are of utmost importance in the treatment of knee OA. Treatments offer limited symptomatic effect and are associated with significant side effects.

There is no cure for this disease despite the availability of a large number of therapeutic options, including non-pharmacological, pharmacological and surgical treatments.[6]

Methotrexate (MTX) is widely used in the treatment of all inflammatory rheumatic diseases, where it seems to act primarily through a mechanism to reduce inflammation.[7]

Imaging studies have established that synovitis is common in OA, supporting the suggestion that inflammation may be important in both peripheral nociception and response to anti-inflammatory treatment.[8] Accordingly, the aim of this study was to assess the efficacy of MTX in decreasing pain and inflammation in symptomatic knee OA.

PATIENTS AND METHODS

Study design

A double-blind randomised placebo-controlled clinical trial comparing MTX with placebo in patients with primary knee OA was conducted at the main university hospital of our institution. The study protocol was approved by the ethics committee of our institution and was conducted in accordance with the principles of the Declaration of Helsinki and its amendments (2008). Informed consent was obtained from all patients before the start of the study.

Patient selection and eligibility criteria

A total of 196 outpatients with knee OA, mainly in tertiary care, from the clinics of the rheumatology and orthopaedics departments were screened. One hundred and forty-four older adults with primary knee OA diagnosed according to the clinical and radiological criteria of the American College of Rheumatology[9] were enrolled in this study. At the screening visit, after providing informed consent, each participant was interviewed and proceeded to washout if they fulfilled the inclusion criteria and had none of the exclusion criteria.

Inclusion criteria

Patients with clinically symptomatic primary knee OA, with persistent knee pain, with clinical signs of synovitis and a disease severity graded moderate to

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severe based on the Kellgren–Lawrence radiographic system[10] were recruited. Symptomatic primary knee OA was defined as knee pain while standing, walking and/or during motion for at least 25 of the 30 days before study entry. Persistent knee pain was defined as >40 mm on a visual analogue pain scale (VAS)/daily pain during the month before study enrolment despite receiving maximum tolerated doses of conventional drugs (including paracetamol 4 g/day and/or a non-steroidal anti-inflammatory drug (NSAID) in the target knee (defined as the most symptomatic knee at study entry). Clinical signs of synovitis were warmth, joint margin tenderness, swelling or effusion.

Recruitment of participants took place over 4 months.

Exclusion criteria

Participants were excluded if they had secondary arthritis related to systemic inflammatory arthritis, including rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, traumatic arthritis, post-infectious arthritis and crystal arthropathies as diagnosed by erythrocyte sedimentation rate, C-reactive protein, rheumatoid factor and synovial fluid analysis. Patients treated with chondroitin sulfate, glucosamine, avocado soybean unsaponifiables or immunomodulatory drugs in the previous 90 days were excluded. Previous joint surgery and administration of oral corticosteroid treatment, intra-articular steroid or hyaluronan injections into the knees within 6 months of screening were also exclusion criteria. Patients with creatinine clearance <75 mL/min, aspartate and alanine aminotransferase levels greater than twice the upper limit of normal, active or recent hepatitis or cirrhosis and patients with serious cardiac or respiratory diseases were also excluded.

Randomisation and treatment allocation

Patients meeting the eligibility criteria were randomised using a computerised random number list in a 1:1 ratio—72 received up to 25 mg/week of oral MTX and 72 received placebo for 28 weeks. Treatment was started with MTX 10 mg/week, increasing to 25 mg/week after 3 weeks. Patients were instructed to take the assigned drug once a week until the end of the study. Folic acid supplementation at a dosage of 5 mg three times a week was also given to both groups. Both the investigators and participants were blinded to the allocated treatment for 28 weeks. All study case report forms recorded only the randomisation number to identify the patient. The investigators were provided with a set of individual sealed decoding envelopes each corresponding to a treatment number. Patients were allowed to continue their usual pain medication provided they did not start any new treatments for their knee OA during the study. All patients received a chart to record the amount of analgesics taken daily, and the use of rescue treatment during the previous weeks was recorded at each study visit. Patients were asked to bring details of all drugs used and unused study drugs at each visit to assess compliance and use of rescue drug change.

Clinical assessment

Clinical assessment consisted of questioning about number of flares, pain, concomitant diseases and analgesics; physical examination for signs of inflammation and primary and secondary measures of disease assessment. All patients had at least two of the following four clinical signs of inflammation: warmth over the joint area, joint margin tenderness, synovial effusion, soft tissue swelling around the knee. An experienced rheumatologist, blinded to treatment assignment, performed the clinical assessments. All assessments were performed at screening, baseline and monthly up to 28 weeks. There was a 24 h washout of analgesic drugs before each visit for symptom assessment and clinical evaluation.

Ultrasound assessment

Ultrasound (US) examinations were performed by a rheumatologist experienced in musculoskeletal sonography using an Antares Sonoline ultrasound device (Siemens Medical Solutions, Mountain View, California, USA) with a multifrequency linear array transducer (6–13 MHz). Maximal depth of effusion and synovial hypertrophy was measured in millimetres. The sensitivity of power Doppler in detecting synovial inflammation depends greatly on the machine used and thus we evaluated synovitis±effusion (synovial membrane) using B mode US only. Intraobserver variability of the US features was tested by performing a second US scan in 25% of the patients, chosen randomly on the same day. Interobserver agreement on US features was done by rereading the stored images. The intra- and inter-reader reliability of US assessments were calculated using K_w.[11] The intraobserver variability using K_w was 0.80 for suprapatellar synovitis, 0.78 for suprapatellar effusion, 0.78 for lateral compartment synovitis, 0.74 for protrusion of medial meniscus, 0.75 for medial compartment synovitis and 0.81 for marginal osteophytes. A K_w value >0.8 indicates excellent reliability.

Outcome measures

The primary outcome criterion defined a priori was pain reduction using a VAS 0–100 mm during the previous 48 h. Secondary outcome measures included improvements in physical function scores and reduction in the degree of inflammation as assessed clinically and by US. Improvements in physical functioning were assessed using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)[12] subscores for pain, stiffness and function, the patient global assessment of the severity of knee OA measured on a 0–100 mm VAS. The physician-reported disease activity VAS was also recorded. Participants were asked if they needed assistance in performing activities of daily living (ADL) tasks (bathing, grooming, dressing, eating, transferring from bed to chair and toileting) using a modified version of the Katz activity of daily living scale.[13] ADL items were scored using an ordinal scale representing level of help and difficulty. These scores were then summed across ADL items to create an ADL disability score (0–12), with a lower score indicating better function.

Data were collected at baseline and monthly up to 28 weeks to determine any change in results from baseline.

Safety evaluation

Safety and tolerability of treatment were assessed at each visit. Safety was assessed by identifying adverse events first occurring or worsening during treatment using open-ended questions and a checklist, including common oral MTX side effects; by physical examination assessing skin, mucous membranes and chest and by laboratory assessment, which included a complete blood count and liver function tests. Adverse events were recorded at each visit and up to 28 weeks and were analysed for their seriousness, intensity and causal relationship with treatment and outcome.

Statistical analyses

Analyses were conducted using SAS V.9.1 (SAS Institute, Inc, North Carolina, USA). To detect a clinically important difference of 20 mm for severity of pain on a 100 mm VAS pain scale between patients treated with MTX and placebo, with 90% power, significant at the 5% level and allowing for a 10% drop-out/loss to follow-up, it was calculated that a sample size of 70 patients was required in each treatment group. Statistical

analyses were performed as intention-to-treat analysis. To assess the impact of missing data multiple imputation was performed. This method reduces possible bias caused by non-completers.[14] A small number of imputations of the missing values was created (M=5) to limit the analysis burden. Accordingly, five imputed datasets were created. Variables for inclusion in the imputation model included demographic data and measures of pain and function. The reasons for withdrawal were prespecified as lack of efficacy, not willing to continue, inability to attend, non-compliance, serious systemic toxic effects or erroneous inclusion. Treatment response was defined by the Outcome Measures in Rheumatology Clinical Trials and Osteoarthritis Research Society International (OMERACT- OARSI) 2004 criteria.[15] Patients were classified as responders if the pain or physical function score decreased by ≥30% and by at least 20 mm on the VAS. Demographic characteristics of the treatment and placebo groups were summarised by descriptive statistics. Potential confounders examined included baseline age, gender, body mass index, comorbidity using the Charlson comorbidity index,[16] which includes diabetes mellitus and cardiovascular morbidity among other disorders. Change from baseline to end point between groups was analysed using analysis of covariance. Estimates of intervention effects were obtained at each follow-up observation. The term 'significant at level 5%' used throughout this manuscript denotes statistical significance. All tests of hypotheses and reported p values are two sided.

RESULTS

Patient population

One hundred and ninety-six people were screened for eligibility during a 4-month recruitment period (figure 1). Of these, 52 (27%) were ineligible and thus 144 patients were enrolled in the study. One hundred and thirty patients (90%) completed the study. The demographic and clinical variables of the two study groups were similar at baseline (table 1). Overall, most of the patients (88%) were women, the mean age was 66 years, with a mean body mass index of 28, mean disease duration of 6 years, 86% used NSAIDs and 88% used paracetamol. Most patients were Kellgren–Lawrence grade II and III.

Efficacy

Results for efficacy end points are summarised in tables 2 and 3. After 28 weeks, patients with symptomatic knee OA who received MTX had significantly greater improvement in both primary and secondary efficacy measures than patients who received placebo.

Primary end point

Knee OA VAS pain scores decreased significantly in the MTX group from 66.7 (13.5) to 40.5 (15.6) mm at 28 weeks compared with the placebo group 66.5 (13.8) to 51.7 (19.6)—that is, the change from baseline was −26.2 versus −14.8 and the mean difference between treatment arms 95% CI was 11.4 (2.8 to 20.0), p=0.009 (table 2). The proportion of patients who had a reduction in VAS of >20 mm was significantly higher in the MTX group (n=38) 53% than in the placebo group (n=17) 24%, p=0.018.

Secondary end points

Table 2 shows the results of the secondary end points in the intervention and placebo groups. There was a clinically relevant reduction in the intervention group compared with the placebo group for scores on all WOMAC subscales at 28 weeks. There was a clinically relevant reduction in ADLs, physician's and patient global assessment of the severity of knee OA at 28 weeks in patients receiving MTX compared with those receiving placebo (table 2).

There was a significantly greater reduction in synovial thickness in the MTX group than in the placebo group as detected by US at 28 weeks, with a change from baseline in total synovial thickness and a change in total effusion score, respectively (table 2).

There was a clinically significant reduction in the percentage of analgesic (NSAID and paracetamol) users in the MTX group compared with the placebo group at 28 weeks compared with baseline ((51/72) 71% vs (63/72) 88%, p=0.012). The

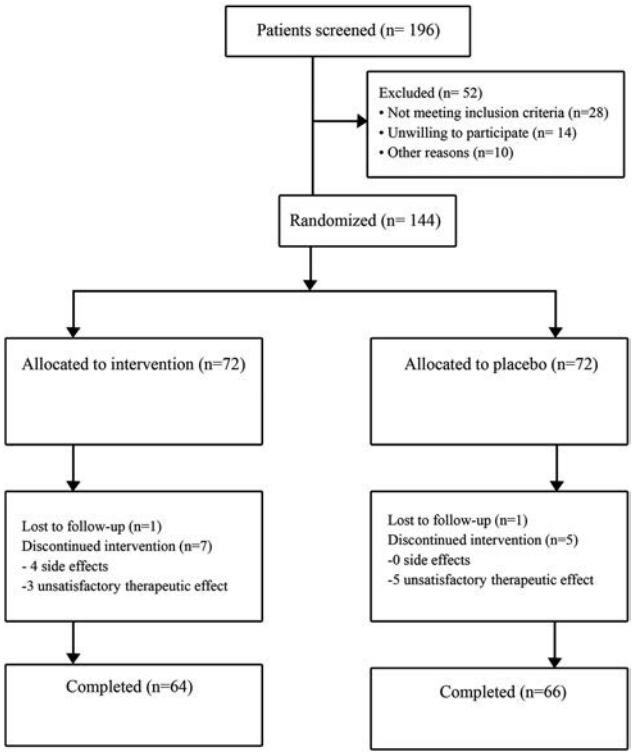

Figure 1 Study population progress.

Table 1 Baseline demographics and clinical characteristics of the study group

Characteristics	Methotrexate (n=72)	Placebo (n=72)
Age (years), mean (SD)	66.5 (5.3)	66.0 (5.5)
Sex (F/M)	64/8	63/9
BMI (kg/m^2), mean (SD)	27.8 (4.5)	27.6 (4.3)
Disease duration (years), mean (SD)	6.1 (4.8)	6.3 (4.6)
Comorbidity, mean (SD)	0.45 (0.78)	0.45 (0.81)
Kellgren–Lawrence grade, n (%)		
II	20 (28)	21 (29)
III	39 (54)	38 (53)
IV	13 (18)	13 (18)
Clinical synovitis, n (%)	72 (100)	72 (100)
Analgesic use, n (%)		
Paracetamol use, n (%)	64 (89)	63 (88)
NSAID use, n (%)	63 (88)	61 (85)

None of the characteristics showed a statistically significant intergroup comparison.
Values are given as mean (SD) or number (percentage).
BMI, body mass index; NSAID, non-steroidal anti-inflammatory drug.

Table 2 Outcome measures at baseline and 28 weeks

Measure	Mean (SD) baseline methotrexate	Mean (SD) change methotrexate	Mean (SD) baseline placebo	Mean (SD) change placebo	Mean difference in change (95% CI)
VAS (0–100 mm)	66.7 (13.5)	−26.2 (−14.7 to −37.7)	66.5 (13.8)	−14.8 (−4.2 to −25.4)	11.4 (2.8 to 20.0) p=0.009
WOMAC pain score (0–20)	10.1 (6.6)	−3.3 (−0.9 to −5.7)	10.0 (6.8)	−1.4 (−0.7 to −2.1)	1.9 (0.7 to 3.1) p=0.002
WOMAC function score (0–68)	42.7 (11.0)	−12.2 (−9.5 to −14.9)	43.5 (11.7)	−4.0 (−0.7to −7.3)	9.5 (3.7 to 15.3) p=0.001
WOMAC stiffness score (0–8)	6.6 (2.6)	−2.1 (−0.9 to −3.3)	6.5 (2.5)	−0.7 (−0.1 to −1.3)	1.4 (0.5 to 2.3) p=0.002
Patients' global assessment (0–100 mm VAS)	62.6 (20.2)	−19.1 (−10.2 to −28.0)	62.5 (19.9)	−10.7 (−2.1 to −19.3)	8.4 (5.3 to 11.5) p=0.000
Physicians' global assessment (0–100 mm VAS)	60.1 (19.1)	−18.4 (5.9 to 30.9)	60.0 (19.5)	−9.9 (−3.0 to −16.8)	8.5 (3.7 to 13.8) p=0.001
Activities of daily living, range 0–10	7.6 (1.9)	−2.5 (−0.2 to −4.8)	7.5 (2.1)	−1.3 (−0.1 to −2.5)	1.2 (0.1 to 2.3) p=0.032
Synovial thickness (mm), mean (SD)	5.6 (4.5)	−1.4 (−0.5 to −2.3)	5.5 (4.6)	−0.4 (−0.02 to −0.78)	1.0 (0.4 to 0.6) p=0.000
Knee effusion depth (mm), mean (SD)	6.9 (6.0)	−1.3 (−0.1 to −2.5)	6.9 (6.2)	−0.8 (−0.05 to −1.55)	0.5 (0.01 to 0.99) p=0.045

Values are given as mean (SD).
VAS, visual analogue scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

OMERACT-OARSI responder rate was (n=38) 53% in the MTX group compared with (n=24) 33% in the placebo group, p=0.018.

Safety

No severe life-threatening events were recorded during the study. There was a higher incidence of mucositis, alopecia, gastrointestinal intolerance and transaminitis in the intervention group compared with the placebo group, but these differences did not reach statistical significance. No serious adverse events such as haematopoietic suppression, hepatic or pulmonary toxicity occurred (table 3). Routine laboratory investigations did not disclose any significant abnormalities in metabolic functions or system organs in either of the two groups (data not shown).

DISCUSSION

OA is now recognised as a disease with an inflammatory component, if not a disease driven by proinflammatory cytokines. Signs of inflammation are common in OA.[2] There is a growing interest in defining the role inflammation plays in OA, which is often associated with low-grade synovitis.

Table 3 Treatment-related side effects

Side effects	Methotrexate (n=72)	Placebo (n=72)
Mild and self-limiting		
Mucositis	2 (2.8)	0 (0)
Alopecia	2 (2.8)	0 (0)
Gastrointestinal intolerance	3 (4.2)	1 (1.4)
Transaminitis	5 (6.9)	1 (1.4)
Severe		
Haematopoietic suppression	0 (0)	0 (0)
Hepatotoxicity	0 (0)	0 (0)
Pulmonary toxicity	0 (0)	0 (0)

Values are given as number (%).

In end-stage OA, synovitis is commonly seen at joint replacement surgery and has even been described as resembling rheumatoid arthritis pannus with new blood vessel formation.[4] It is thus feasible to suppose that MTX may be useful in OA.

This study demonstrated that in patients with symptomatic knee OA with clinical evidence of synovitis, a 28-week course of oral MTX was better than placebo in reducing pain and clinical synovitis and US-detected synovitis as shown by a reduction in the primary and second outcome measures. A significant reduction in pain measured by the VAS in the MTX-treated group compared with the placebo-treated group was demonstrated at 28 weeks. In addition, there was a significant improvement in pain as measured by the WOMAC pain subscale in the MTX group compared with the placebo group. A previous smaller study of 16 weeks' duration demonstrated reduction in pain and improvement in synovitis and physical function.[17]

Two open-label studies have demonstrated improvement in OA pain with MTX use. One of these, a study for erosive hand OA, showed a significant improvement in pain with 10 mg MTX.[18] Recently, an open-label study, using MTX for pain relief in knee OA, reported that a high proportion of patients had considerably reduced pain comparable to that achieved with NSAIDs and opioids. MTX had an analgesic effect in patients with moderate knee pain.[19] In the same study no correlation was noted between change in imaging and change in pain scores at the end of the study. The authors attributed this lack of correlation possibly to the small number of participants, all of whom had moderate to high pain scores.[19]

Synovitis is thought to play a dominant role in the development of pain, joint inflammation and cartilage destruction in OA.[2 3] In this study, all patients selected had clinical synovitis. The hypothesis is that the analgesic response to MTX seen in OA is mediated through anti-inflammatory action on the synovium. Some evidence to support this hypothesis is the improvement in clinical and US-detected synovitis in the MTX-treated group compared with the placebo-treated group. In patients with OA, it is recognised that low-grade synovitis is often present and may correlate with pain severity.[2 3] Indeed,

Abou-Raya A, *et al*. *Ann Rheum Dis* 2014;**77**:e46. doi:10.1136/annrheumdis-2013-204856

inflammation may be the crucial link between noxious stimuli and recruitment of centrally mediated pathways.

The association between synovitis and pain indicates that inflammation may have a pivotal role in causing knee pain. Inflammatory mediators play a pivotal role in the three most recognised phenotypes, among which is the ageing phenotype. Furthermore, it is suggested that inflammation drives OA as shown by the existence of flares in OA, which often resemble those in other types of inflammatory arthritis, characterised by nocturnal pain, stiffness and swelling.

The intention-to-treat analysis showed improvement for the MTX group on all WOMAC subscales, including pain, stiffness and function. Pain is the over-riding clinical problem in OA and treatments are often ineffective. Mechanisms of pain in different subsets of OA are different and complex. MTX seems to alleviate pain in the inflammatory phenotype of OA. It helps to decrease synovitis in many inflammatory joint diseases, particularly rheumatoid arthritis.[20] Several lines of evidence suggest that some pathological changes seen in the synovial inflammation of rheumatoid arthritis are also seen in OA.[4 21]

The findings of this study indicate that MTX may have dual benefit—improving pain and function, both of which influence quality of life. Improvement in WOMAC scores which reflect function, were particularly striking in patients receiving MTX compared with those receiving placebo. WOMAC scores measure physical function and their improvement indicates better functioning ability due to less pain, stiffness and/or fewer functional limitations. When interpreting the results of physical function, one needs to keep in mind the progressive nature of OA. Thus, an improvement in WOMAC scores or even a stable measure of physical function represents a treatment success. This may in itself justify the use of MTX in patients with OA. It is noteworthy, however, that the control group also improved during follow-up.

The reduction in pain and improvement of function together with the improvement in clinical and US-detected synovitis in the MTX group suggest that signs of inflammation in patients with OA may identify an OA subset or phenotype rather than merely reflect a stage of disease progression.

Safety analysis confirmed that MTX has few, and mostly well-tolerated, side effects. No severe or serious adverse events were noted.

This is the first randomised placebo-controlled trial conducted to determine the efficacy of MTX in knee OA; however, the results of this study should be interpreted in light of several limitations. One is the sample size, two is the relatively short duration of the study and the difficulty in interpreting changes evaluated as means rather than as individual improvements. Another limitation is that owing to the different phenotypes in OA, MTX may be more effective in certain subgroups of OA—namely, the predominantly inflammatory phenotype. Further larger, longer-term trials are required to fully assess the safety and efficacy of MTX over a period of time that is more reflective of clinical practice.

CONCLUSIONS

In conclusion, the results of this study provide evidence for the efficacy and tolerability of MTX in reducing pain and subsequently improving function in older adults with knee OA. In an attempt to optimise pain management and reduce analgesic use, MTX may provide an effective and welcome addition to the management of certain subsets of knee OA.

Correction notice This article has been corrected since it was published Online First. The below **Expression of concern** statement has been included.

Contributors AA-R participated in the trial design, conduct of the trial and manuscript writing. SA-R participated in the trial design, conduct of the trial and manuscript preparation. TK participated in the trial design, conduct of the trial and data analysis.

Competing interests None.

Patient consent Obtained.

Ethics approval Institutional review board of the Faculty of Medicine, University of Alexandria.

Provenance and peer review Not commissioned; externally peer reviewed.

Expression of concern 5 May 2016: We wish to express concern about the reliability of data in this paper because of errors in the results due to mistakes in the data files. Following publication, analyses of data was provided by the authors and a subsequent investigation was undertaken by the ethics committee of the faculty of medicine of Alexandria University. The university's investigation concluded that there was an unintentional mistake in the statistical process with errors in collection of data in some groups. Corrected information has been provided and is being reviewed by the journal.

REFERENCES

1 Hunter DJ, Felson DT. Osteoarthritis. *BMJ* 2006;332:639–42.
2 Sellam J, Berenbaum F. The role of synovitis in pathophysiology and clinical symptoms of osteoarthritis. *Nat Rev Rheumatol* 2010;6:625–35.
3 Samuels J, Krasnokutsky S, Abramson SB. Osteoarthritis: A Tale of Three Tissues. *Bull NYU HospJt Dis* 2008;66:244–50.
4 Shibakawa A, Aoki H, Mauko-Hongo K, *et al*. Presence of pannus-like tissue on osteoarthritic cartilage and its histological character. *Osteoarthritis Cartilage* 2003;11:133–40.
5 Pelletier JP, Martel-Pelletier J, Abramson SB. Osteoarthritis, an inflammatory disease: potential implication for the selection of new therapeutic targets. *Arthritis Rheum* 2001;44:1237–47.
6 Zhang W, Moskowitz RW, Nuki G, *et al*. OARSI recommendations for the management of hip and knee osteoarthritis. Part 1: Critical appraisal of existing treatment guidelines and systematic review of current research evidence. *Osteoarthritis Cartilage* 2007;15:981–1000.
7 Ranganathan P, Brinker RR. Methotrexate pharmacogenetics in rheumatoid arthritis. *Clin Exp Rheumatol* 2010;28(Suppl 61):S33–9.
8 Conaghan PG, Rhodes L, Hensor EM, *et al*. MRI study of the extent of 'Gold-Standard' evaluated synovitis and its relationship to pain in osteoarthritis of the knee. *Arthritis Rheum* 2006;5(Suppl):S822.
9 Altman R, Asch E, Bloch D, *et al*. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. *Arthritis Rheum* 1986;29:1039–49.
10 Kellgren JH, Lawrence JS. Radiological assessment of osteoarthrosis. *Ann Rheum Dis* 1957;16:494–502.
11 Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977;33:159–74.
12 Bellamy N, Buchanan WW, Goldsmith CH, *et al*. Validation study of WOMAC: a health status instrument for measuring clinically important patient relevant outcomes to antirheumatic drug therapy in patients with osteoarthritis of the hip or knee. *J Rheumatol* 1988;15:1833–40.
13 Katz S, Akpom CA. A measure of primary sociobiological functions. *Int J Health Serv* 1976;6:493–508.
14 Molenberghs G, Kenward MG. Missing data in clinical studies. Chichester: John Wiley & Sons; 2007.
15 Pham T, van der Heijde D, Altman RD, *et al*. OMERACT-OARSI initiative: Osteoarthritis Research Society International set of responder criteria for osteoarthritis clinical trials revisited. *Osteoarthritis Cartilage* 2004;12:389–99.
16 Charlson M, Szatrowski TP, Peterson J, *et al*. Validation of a combined comorbidity index. *J Clin Epidemiol* 1994;47:1245–51.
17 Abou-Raya S, Abou-Raya A, Sallam N. Methotrexate: A therapeutic option in symptomatic knee osteoarthritis: randomized placebo-controlled trial. *Ann Rheum Dis* 2012;71(Suppl3):415.
18 Pavelka K. Methotrexate in the treatment of erosive OA of the hands. *Ann Rheum Dis* 2006;65:402.
19 Wenham CYJ, Grainger AJ, Hensor EM, *et al*. Methotrexate for pain relief in knee osteoarthritis: an open label study. *Rheumatol* 2013;52:888–92.
20 Cronstein B. How does methotrexate suppress inflammation? *Clin and Experimental Rheumatol* 2010;28(Supp 6):S21–3.
21 Scanzello CR, McKeon B, Swain BH, *et al*. Synovial inflammation in patients undergoing arthroscopic meniscectomy: molecular characterization and relationship to symptoms. *Arthritis Rheum* 2011;63:391–400.